Preventing hepatitis B and hepatitis A in an area of low endemicity

van Steenbergen, J.E.

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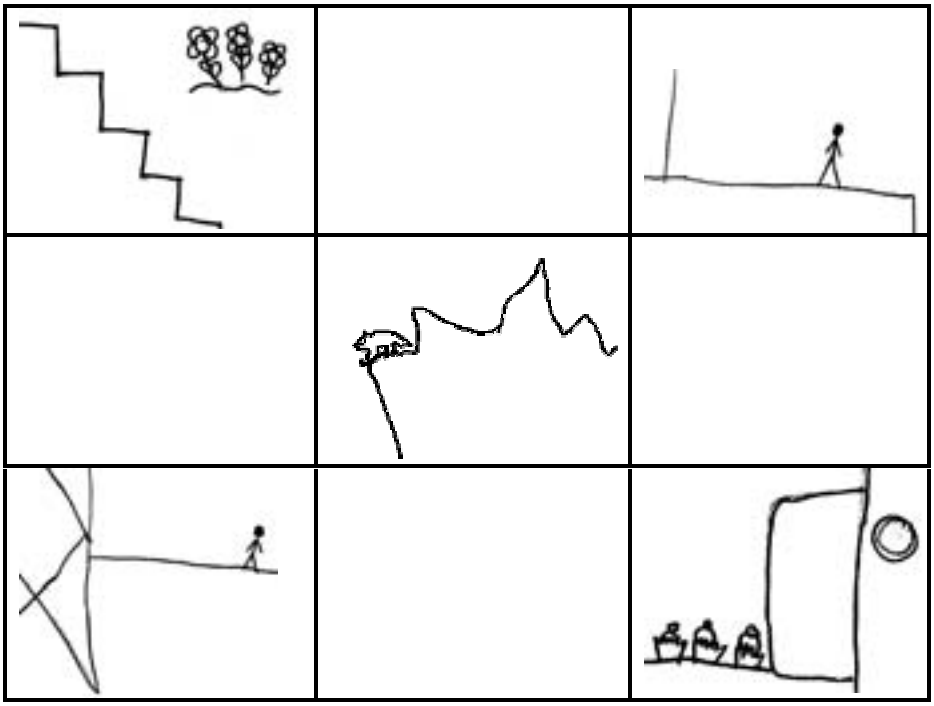

Illustrated by Jonah van Steenbergen

J.E. van Steenbergen

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Preventing hepatitis B and hepatitis A in an area of low endemicity

Academisch Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit van Amsterdam op gezag van de Rector Magnificus prof.mr. P.F. van der Heijden ten overstaan van een door het college voor promoties ingestelde commissie, in het openbaar te verdedigen in de Aula der Universiteit

op dinsdag 3 februari 2004, te 12.00 uur

door James Everard van Steenbergen
geboren te Utrecht

Promotiecommissie

promotor: prof.dr R.A. Coutinho, Universiteit van Amsterdam
co-promotor: dr. J.A.R. van den Hoek, afdelingshoofd GG&GD Amsterdam

overige leden: prof. dr. J. van der Noordaa, emeritus Universiteit van Amsterdam
prof. dr. G.J. Bonsel, Universiteit van Amsterdam
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prof.dr. P. van Damme, Universiteit van Antwerpen
dr. M.A.E. Conyn-van Spaendonck, RIVM, Bilthoven

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Most of the research presented in this thesis was conducted at the Department of Infectious Diseases, Municipal Health Service Amsterdam (GG&GD Amsterdam), where the author was employed from January 1999 to December 2003. The studies were funded by the GG&GD. The pilot project, directed at vaccinating groups with behavioural risk against hepatitis B, was funded by the Dutch Ministry of Health. Aventis Pasteur MSD the Netherlands, facilitated the pilot by supplying the vaccines in the control areas free of charge. Coordination of the pilot was conducted at the LCI (National Coordination Communicable Disease control), Utrecht, the Netherlands.

“But however secure and well-regulated civilized life may become, bacteria, Protozoa, viruses, infected fleas, lice, ticks, mosquitoes, and bedbugs will always lurk in the shadows ready to pounce when neglect, poverty, famine, or war lets down defences. And even in normal times they pray on the weak, the very young and the very old, living along with us, in mysterious obscurity waiting their opportunities. About the only genuine sporting proposition that remains unimpaired by the relentless domestication of a once free-living human species is the war against these ferocious little fellow creatures, which lurk in the dark corners and stalk us in the bodies of rats, mice, and insects, and waylay us in our food and drink and even in our love.”

Hans Zinnser (1934), Rats, lice and history. The biography of a bacillus.
page 12

Contents

Acknowledgements

A thesis is written by a lonely scientist, thinking, reading, writing in solitude and he or she emerges from thoughts and studies with “Eureka!”, to immediately start reasoning with other scientists, trying to convince them of the new theory, relevance of new data, and consequences for life or professional practice. This is, however, not what happened during my work at the MHS Amsterdam (GG&GD). In fact, I have done little more than to look at the registries of public health nurses of this and other MHS facilities. Thus, this thesis is a written record of a decade of work as it was conceived by Anna Leentvaar-Kuypers and continued by Anneke van den Hoek, and implemented by an excellent team of public health nurses under guidance of Dorothé Baayen. It was Anna who inspired me to do research, from the first day I entered the MHS Amsterdam as trainee in communicable disease control. I am indebted to her, not only for her natural stimulus in scientific thinking and opportunity to evaluate and investigate communicable disease control, but also for her always critical and witty approach and guidance in research. Anna was always enthusiastic, while Anneke, her succesor, served as a scientific razor blade in reasoning and writing and guided me through the process as compassionate co-promotor. I especially like to mention Jan Lelijveld, who showed me how to bend African experience in motivated high quality Dutch communicable disease control.
The work of public health nurses starts with patients that are reported to the GG&GD. Patients are informed, samples taken, laboratory results collected; patients receive health education, hygienic advice; contacts are listed and traced, and all this work is registered meticulously. With these data most of the analyses were done. Presently, the team of excellent public health nurses and nurse assistants consists of Lian Bovée (co struggle with SPSS), Philippine de Boer (crucial for the antenatal screening and tracing contacts), Karin de Boer, Katinka Burgers (manager of the HAV pilot), Monique Dekker, Sylvia Esman, Evelien Siedenburg (for carrots and antioxidants), Caroline van Gielen, Willeke Hiemstra (age mate), Aukje Keijzer, Joan Kint (kitchen expert), Susan Koeman, Henny van de Laar (at another table), Dieke Mulder (who started the HBV pilot in 1998), Dieuwke Ram, Liesbeth Lanser, Annie Timmerman, Frits Trompetter, Zen Vlek, Desta Nourhussen, Carrolyn Gambier and, best of all, but serious deserter, Alie Westdijk. Special thanks for Gerard Sonder, MD, who had a major contribution in HAV data collection. Room mates are important for social well being, special thanks to Leny Gerber (seasonal sugar intake) and Willy Maruanaya (green tea). Important contributors to the studies are acknowledged by being co-author or at the end of the respective chapters. One co-author features in all studies, Roel Coutinho, promoter of this thesis. Fast, precise, strict, but righteous. I am greatly indebted

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A thesis in the medical field can be a rather redundant piece of writing, as important components are published in scientific journals. I am very happy that this holds not true for this thesis, because the unique illustrations give added value, which no journal can ever attain.

November 2003

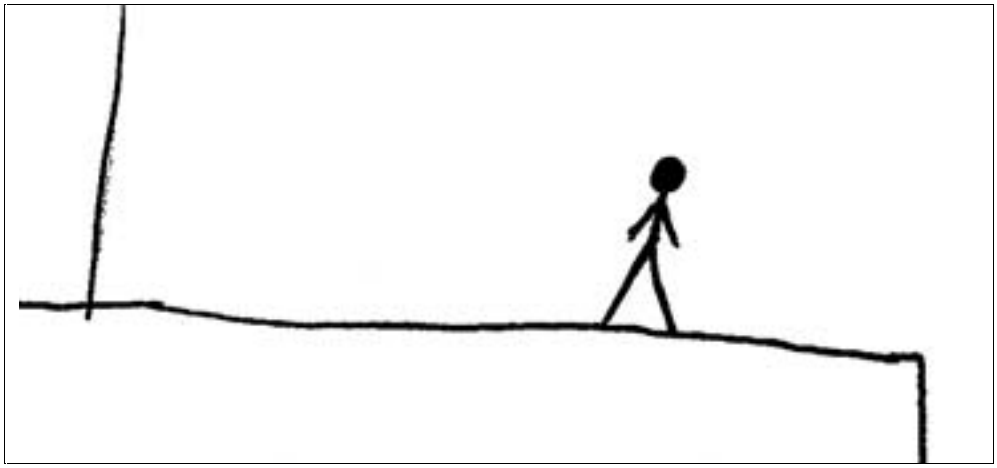
Abbreviations, acronyms

antiHAV-IgM	antibodies to the hepatitis A virus of the immune globulin M type
antiHBc	antibodies to the hepatitis B core antigen
antiHBe	antibodies to the hepatitis B e (Espmark) antigen
antiHBs	antibodies to the hepatitis B surface antigen
CI	confidence interval (in general 95% confidence interval)
CMR	continuous registration of morbidity in general practice
DU	drug-users
ELISA	enzyme linked immuno sorbent assay
FAM	family (acquired HAV from household member)
GGD	Municipal Health Service, Gemeentelijke Gezondheids-dienst
GG&GD	Municipal Health Service, Gemeentelijke Geneeskundige en Gezondheidsdienst
HAV	Hepatitis A virus or hepatitis A
HBeAg	Hepatitis B e (Espmark) antigen
HBIG	hepatitis B immune globulin
HBsAg	hepatitis B surface antigen
HBV	Hepatitis A virus or hepatitis A
HBV-I, HBV-II, HBV-III	vaccine against hepatitis B, first, second and third dose
HIV	human immune deficiency virus
IDU	injecting drug-users
IgG	immune globulin type G
IgM	immune globulin type M
IMP	imported (acquired HAV while abroad)
IU/L	international units per litre
IVF	in vitro fertislisation
MHS	Municipal Health Service (GGD)
MSM	men having sex with men
NCvB	Netherlands Centre for Occupational Diseases, Neder-lands Centrum Beroepsziekten
NIVEL	Netherlands Institute for Health Services Research, Ne-derlands Instituut voor onderzoek van de gezondheid-zorg
OR	Odd's ratio
PCR	Polymerase chain reaction
PHL	Public Health Laboratory, Streeklaboratorium voor de Volksgezondheid

R_0	Basic reproductive rate
RIVM	National Institute for Public health and the Environment, Rijksinstituut voor Volksgezondheid en het Milieu
RT	reverse transcriptase
SCH	school (acquired HAV at school)
SW	sex worker
STD	sexually transmitted disease
STI	sexually transmitted infection
VP	viral protein
WHO	World Health Organisation

Chapter 1

Epidemiology and control of HBV and HAV in Amsterdam and the Netherlands, an area of low endemicity



Abstract

Background- Both hepatitis B and A infections (HBV, HAV) are caused by a virus and are vaccine-preventable. Both viruses are ubiquitous, with high incidence in the developing world; incidence is low in the industrialised world, where these infections are, nevertheless, among the most frequently reported communicable diseases. In the Netherlands each year, 350 individuals are admitted to hospital with illnesses related to HBV, of whom 60 die; 130 are admitted as a consequence of HAV, with one or two deaths. The majority of both infections are silent, however, which contributes in unknown part to their transmission.

Epidemiology- The nationwide incidence of reported acute HBV decreased by more than half since the early eighties, from 6 to 1.2/100.000. In Amsterdam reports similarly decreased in the last decade from highest levels in 1986 and 1993 (8.4/100.000) to lowest in recent years 1997-2002 (2.2-3.7/100.000). The groups at highest risk are men having sex with men (MSM), and heterosexuals with multiple and/or unsafe sexual contacts. Injecting drug users (IDUs) used to be an important risk group, but are not reported anymore. The nationwide incidence of reported acute HAV is currently less than 4.4/100.000. In Amsterdam, the HAV reports decreased since 1998 from average 25/100.000 to recent years in the range of 7 – 14/100.000). Most reported cases come from travellers plus household and school contacts of cases. The number of MSM reports is increasing, but IDUs are not reported with HAV. For both HBV and HAV, in over 20% of cases no probable source can be found.

Control- Universal vaccination seems not cost-effective in either disease. For HBV, the Netherlands relies on a highly targeted vaccination approach, with antenatal screening and neonatal vaccination. This program seems effective, according the results of the evaluations in 1992. People in behavioural risk groups have been advised to seek vaccination, but not systematically covered. Vaccination strategy against HAV is in its infancy. Vaccination is advised for children that regularly travel to countries with intermediate or high endemicity for HAV, but no national policy is implemented. Local initiatives by municipal health service (MHS) facilities try to improve coverage for this group. As for MSM, no policy is implemented at a national or local level.

Aims of the studies- Of the studies presented in this thesis, two evaluate existing HBV vaccination programs (for screening pregnant women and vaccinating their newborns, and household contacts). One evaluates a pilot program directed at behavioural risk groups. For HAV, the present post-exposure policy is evaluated. Three studies describe modern molecular techniques, that, combined with sound epidemiological data, can illuminate unknown patterns of transmission of both agents.

Introduction

Disease burden

- Clinical spectrum
- Hospital and mortality data

Descriptive epidemiolgy

- Notifications, surveys
- Epidemic profile
- Specific risk groups
- Occupational risks
- Travellers
- Unknown source

Control strategies

- Hygienic precautions
- Notification and post-exposure prophylaxis
- Vaccination
  - Targeted high-risk groups
  - Occupational risk groups
  - Travellers
  - Universal

Virological typing

Aims of the studies

Conclusion

Introduction

Hepatitis B and A illness differ little in clinical presentation. Both cause mild, if any, disease in children, but can cause more morbidity at higher ages. Both viruses are ubiquitous, but have low incidence in the rich industrialised western world, while having high incidence in the developing world.

Figure 1 Global prevalence

Figure 1.a HBV

Global endemicity of HBV. Shown in green are areas of HBsAg prevalence exceeding 1%, equalling areas with overall prevalence of HBV markers > 10%

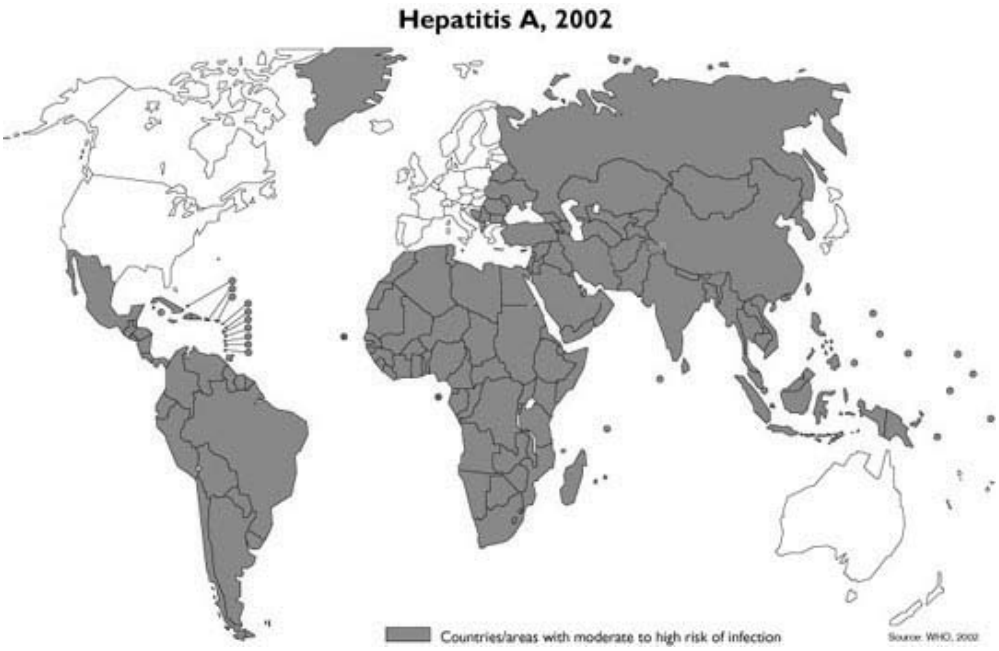
.

Source: www.who.int/ith/chapter05_m04_hepatitisb.html, accessed November 2003

The geographical distribution of serotypes and genotypes:
- A North and Central Europe (Old World, *adw2, ayw1*)
- B East Asia (*adw2*, *ayw1*)
- C Pacific and Asia (*adw2, adrq*$^{+}$)
- D Mediterranean, Middle East (Old World, *ayw3, ayw2*) with spread in Western world
- E West Africa (*ayw4*)
- F South America (*adw4*, *ayw4*)
- G France
- H Central America

Figure 1.b HAV

Global endemicity of HAV. Shown in green are areas of antiHAV prevalence in adolescents exceeding 10%.



Source: /www.who.int/ith/chapter05_m03_hepatitisa.html, accesed November 2003

Geographical distribution of genotypes:	
IA	America's, Asia, Russia, (IA representing 2/3 of all studied strains)
IB	Mediterranean area, Australia, Europe, South America
II	France
IIIA	India, Sri Lanka, Nepal, Malaysia, (and also found in captive owl monkeys)
IIIB	Denmark, Japan
VII	Sierra Leone

Hepatitis B and hepatitis A virus (HBV and HAV) infections are both vaccine-preventable, but the two illnesses differ in historical background, clinical spectrum of disease, causative viruses, mode of transmission, and epidemiology. Thus, they require different control strategies. The simplest approach would be universal vaccination for both, but this is at present infeasible in the Netherlands due to costs, possible side effects, ethical considerations, and societal reluctance to augment the childhood vaccination program.

This thesis addresses the control of HBV and HAV in Amsterdam, The Netherlands, an area of low endemicity. Existing control programmes were studied, a new approach was tested, and modern technology was used to unravel the epidemiology, that should form the basis for future policy decisions. Most of the work was done in the Department of Infectious Diseases of the Municipal Health Service (MHS) Amsterdam, which offers public health nurses that are willing to register their activities routinely in a database and, essential but unique in this country, a public health laboratory within the same organisation. The epidemiological situation in Amsterdam (approximately 735.000 inhabitants) differs slightly from other parts of the country (approximately 16 million inhabitants), but conclusions from our studies apply nationwide, and might benefit areas of low endemicity outside the Netherlands.

Several textbooks fully describe the historical background (1), the virology (2-4), the clinical picture (5), the basic epidemiology (6) and vaccinology (7; 8) of HBV and HAV. As an introduction to the seven studies that form this thesis, a brief description follows as to their burden of disease in the Netherlands, the Dutch epidemiological situation and control strategies, and the basics of typing of these two viral agents. Finally, the aims of the seven studies are presented.

Disease burden of viral hepatitis B and A

HBV clinical spectrum

In most HBV-infected children and adults, symptoms are absent or non-specific. Notification data (i.e, reports to MHS) thus present an incomplete picture of HBV epidemiology. One third of infected individuals develop an icteric phase of 2-6 weeks. Rarely, acute symptomatic HBV infection leads to acute liver failure (1%) and death (0,1%), but more than 95% of adults spontaneously and completely recover (9), while 3-5% remain persistently infected and are at increased risk to develop of cirrhosis and hepatocellular carcinoma over the ensuing decades at the rate of 1/1000 chronically infected/year(10; 11). Clearing the virus is age dependent. In contrast to adults, HBV infection in children can often lead to persis-

tent infection. Infection at birth leads in 95% of cases to persistent infection, compared to 30% if infected ages 0-6 years; adolescents seldom become chronically infected. The liver-related mortality rate for chronically infected HBV patients is around 1/1000 person years. In HIV co-infected individuals, spontaneous recovery from HBV infection is reduced (12; 13), and the liver-related mortality rate is 14/1000 person years (14).

HBV hospital admissions and mortality

Patients with any disease are coded at hospital discharge according to the International Classification of Diseases with a primary and secondary diagnosis. There is a code for HBV, but the coding criteria do not encompass all sequelae of chronic HBV. Several diagnoses might mask HBV-infection, such as hepatic coma, liver cirrhosis, hepatocellular carcinoma, but might equally reflect other pathologies. When the National Institute of Public Health and the Environment (RIVM) analysed data of years 1993-97 (15), it found that each year 165 patients were discharged with a putative primary diagnosis HBV. Another annual 165 were coded as chronic hepatitis, 350 as hepatocellular carcinoma, and 750 as non-alcoholic cirrhosis of the liver (15). Whether these numbers represent new cases or readmissions for the same patient is unknown, as is the percentage of HBV related disease under each code.

The national death registry, for the years 1991-94, recorded an average of 4 deaths/year with acute HBV as primary cause (15); in years 1996-2001 the average was 2.2 (16). From 1996-2001, an annual 30-60 persons (average 43) died following chronic HBV. Similar to hospital data, other causes of death (chronic hepatitis, liver cirrhosis, hepatocellular carcinoma) might mask HBV-infections. These causes of death combined, cause an average 500 deaths annually (15). This total of 500 would be an overestimation of HBV related deaths. The number of deaths is directly related to the number of chronically infected individuals, as indicated above, in a rate of 1/1000 chronically infected person years. The Health Council of the Netherlands estimated size and carrier rate of known groups at high risk of acquiring HBV (17). Based on these estimates, the minimum number of chronic carriers can be estimated at approximately 60.000. These data are far from accurate, but represent a reasonable guess. Correspondingly, 60 annual deaths can be expected, but the true number of HBV-related deaths remains unknown.

HAV clinical spectrum

Age is the most important factor related to clinical expression. Of children 5 years and younger, 70% have undetected or non-specific symptoms (70%), and infection often goes unrecognised; 30% show fever or dark urine, with or without

jaundice (18). Adults generally have symptoms (over 90% of infections), and over 75% of infections in adults become icteric (19). Less than 1.5% of hospitalised patients develop fulminant hepatitis, which has a high death rate (70-90%). Case fatality of all reported HBV-infections is low for ages 15-40 years (< 0.4%) but 2.7% for ages 40 years and older. Since most data are biased towards clinical signs, the morbidity data might be an overestimation. Chronic sequelae do not occur with HAV, but complete recovery may take several months, especially among adults.

HAV hospital admissions and mortality
RIVM analysis of data from hospital records 1993-1997 estimated an annual average of 130 hospital admissions (20). In the last six years, 1996-2001, the national death registry recorded 0 - 3 deaths annually caused by acute HAV, with an average of 1.3 (16).

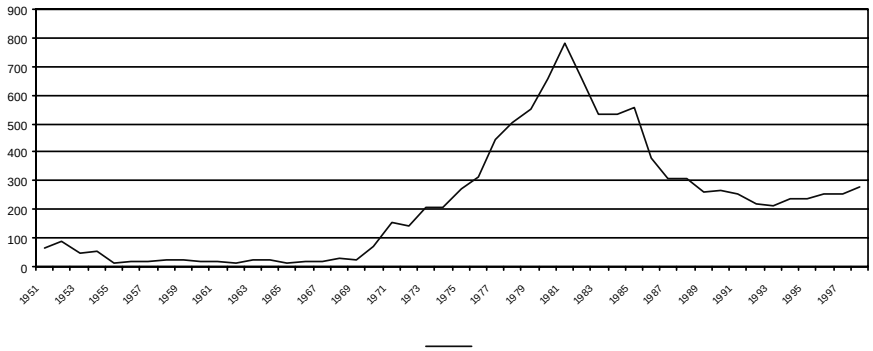
Descriptive epidemiology of viral hepatitis B and A

Introduction
Reporting cases of HBV and HAV infection is obligatory in most countries of the western world, and both are among the most frequently reported communicable diseases. For both, the true number of new infections is not reflected by reported cases, as most infections go unnoticed, and reporting of symptomatic cases is often forgotten. Other means of surveillance is needed to get a clear picture on the epidemiology of both diseases. The Netherlands is an area of low endemicity for both infections, as are the Scandinavian countries, while endemicity is intermediate in southern countries of Europe. High endemicity is found in the developing world.

Acute HBV infections
In the Netherlands since 1951, acute HBV is an MHS-notifiable disease, reported as “serum hepatitis”. Distinction in serum hepatitis and infectious hepatitis was made on epidemiological criteria. In the late sixties the laboratory test for Australia antigen became available (later acknowledged to be HBV surface antigen, HBsAg).
Since April 1999, chronic hepatitis must be reported as well. The RIVM found, that pre-1999 data were confounded by erroneous reporting. Over the years 1995-98, 12% of the reported cases of acute HBV infections concerned chronically infected patients; in another 33%, the true state, acute or chronic, was unknown.

Figure 2HBV

Annual HBV notifications to the MHS in the Netherlands, 1951-1998 (truncated in 1999 as, since then, chronic and acute were both notifiable).

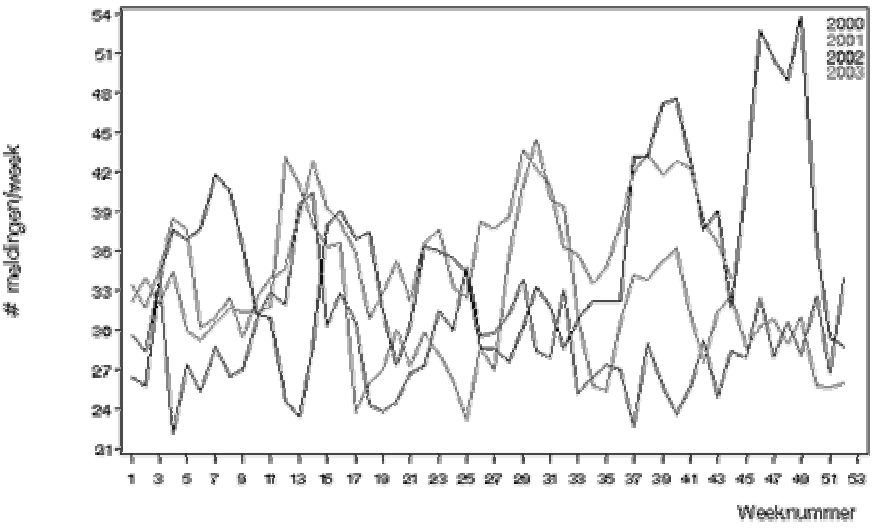


source: IGZ/RIVM

Figure 3 Seasonal variation of viral hepatitis B and A in The Netherlands

Figure 3HBV

Seasonal patterns HBV, based on number of MHS reports per week (#meldingen/week) to the Health Inspectorate for the years 2000-2003



source: www.rivm.nl/isis, accessed November 17, 2003

Across the Netherlands, between 1993 and 1998, the reported incidence of MHS notifications for HBV varied from 1.4 to 1.8/100.000 inhabitants (21), but a general practitioners' sentinel system suggests that the actual HBV incidence was two to six times higher, ranging from 3.2 – 6.1/100.000 (22). In the last decade, the annual number of notifications declined to, at present, under 200. In Amsterdam, the notifications similarly decreased in the last decade, from their highest levels in 1986 and 1993 (8.4/100.000) to their lowest years in recent years 1997-2002 (2.2-3.7/100.000). The groups with behavioural risk of acquiring HBV, drug users and MSM, contribute most to the decline in Amsterdam. No specific cycles were seen (Figure 2), and a seasonal pattern was not detectable (Figure 3).

Serological surveys

The RIVM analysed 7395 sera collected in 1996 from a sample of the population ages 17-79 years. Serological markers of HBV infection were found in 2.1%, and markers of chronic infection were found in 0.2%; both increased with age (23; 24). There was no information on specific HBV-risk behaviour. In 1991, the first evaluation of nationwide antenatal screening showed a prevalence of 0.44% chronically infected pregnant women (25). Amsterdam analysed sera of its antenatal screening in the same period and found 1.2% chronically HBV infected women (26). More recently, in 2000-2001, national antenatal screening data showed 0.37% carriers (27). Seroprevalence in non-participating women was higher, compared to participating women (28; 29).

MATHEMATICAL MODELLING

The RIVM developed a dynamic mathematical HBV transmission model, with exclusion of injecting drug users (IDU) and transmission to non-users who are their sex partners (30). The annual average number of new infections was calculated at 2.250, leading to 220 new chronic carriers every year (31).

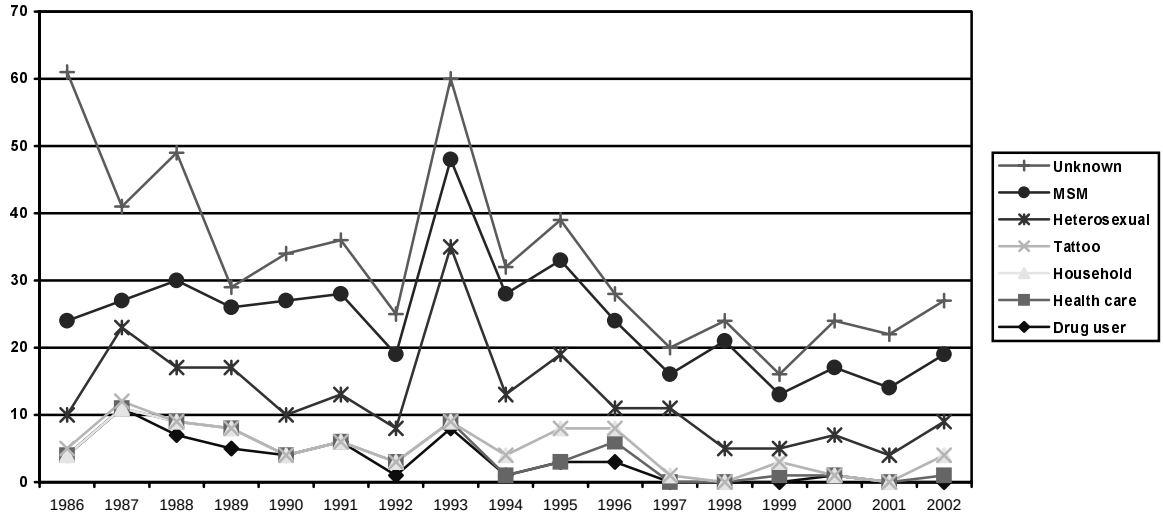
HBV epidemic profile

Epidemics in the general Dutch population are unusual, but outbreaks frequently occur, most often related to breaches in infection control in healthcare settings. In the last decade, outbreaks occurred related to in vitro fertilisation (32), to endocardial biopsies (33), and to a chronically HBV infected surgeon (34). Further small clusters have been related to transmission incidents in schools, beauty parlour, tattoo shop (35). Epidemics among IDUs are described as early as the 1970s (36). The Utrecht MHS was confronted in 1983 with an outbreak in tinkers travellers, a non-immigrant group that traditionally lives in mobile homes (37). Among the 252 tinker travellers, 30 HBV carriers were found (12%). Ensuing

study ruled out an epidemic but indicated public health confrontation with a high HBV-endemic sub-population that had been living unnoticed in the low-endemic Netherlands. Nation-wide research later, showed that HBV is not a problem for tinkers overall but only in certain large tinker families (38).

Figure 4HBV

Annual notifications to the MHS of acute symptomatic HBV in Amsterdam, 1986-2002



source: annual reports Dept. Infectious Diseases, MHS Amsterdam

HBV specific risk groups

Household or sexual contacts of infectious individuals (especially chronic carriers) and children of carrier mothers are at increased risk for acquiring HBV infection. Family members of adoptees with chronic infection are at similar risk. The data of a RIVM co-ordinated national two-year enhanced surveillance project are not yet analysed as to the source person. However, in Amsterdam, since 1986, only in 2.3% of notifications a source is found within the household (Figure 4). Seeking healthcare as a patient can also pose a risk for HBV, as was seen in the past for frequent recipients of blood or blood products (haemophiliacs) or for those involved in regular percutaneous procedures (hemodialysis). In the Netherlands, such persons are no longer seen in the notification registry, due to reduction of these risks.

In the national registry of reported HBV cases, 25% are transmitted heterosexually and 30% homosexually (39). In the last twenty years, the MHS Amsterdam registered similar data: 23% heterosexual and 35% homosexual (40; 41). In the early 1980s, injecting drug use was an important risk factor for HBV. Seroprevalence studies among IDUs in various parts of the country have indeed shown an increased prevalence of serological markers of previous infection: Rotterdam 61%, Heerlen/Maastricht 67%, The Hague 35%. (42). In Amsterdam, a 1990s seroprevalence study showed markers in 67% of IDUs (43). However, while jaundiced IDUs continue to be referred to the MHS, HBV was confirmed only once in years 1997-2002 (40; 41). Previously, over 12% of its HBV cases were IDU. The national registry of notified cases likewise saw a decline in annual number of drug users since the mid-1990s, from an annual 10% of cases to presently less than 1% (39).

As IDUs frequently are incarcerated, prisoners are regarded as high-risk groups for acquiring HBV, but in Dutch prisons, no transmission has been recorded. Another group not seen in the registry, despite increased risk, are those living in an institute for mentally disabled (44), including staff. Their risk reflects the type and frequency of intimate contacts and also the number of carriers, since especially individuals with Down syndrome are predisposed for carriership if infected.

HBV occupational risks

Cases reported to the MHS should mention the patients occupation but these data are not transferred to the national registry. There is an obligatory reporting of occupationally acquired diseases to the Ministry of Social Affairs, but the registry is far from complete. Occupational health services voluntarily report occupational diseases to the Dutch Centre for Occupational Diseases (NCVB). From April 1997 to September 2003, 16 cases of occupationally acquired HBV were reported (45), of whom 12 were in healthcare. In the MHS Amsterdam, 2.3% of reported HBV were related to healthcare, either as a patient or staff. In policemen, fire fighters, and other public safety workers, no increase of prevalence of serological markers has been found (46). In an annual average 150 needle stick or other percutaneous accidents reported to the MHS Amsterdam, blood-borne pathogens might have been transmitted, but in 1886 such accidents over ten years, HBV was never transmitted (47). The same goes for workers in sewage or refuse disposal: high risk but few cases. As indicated above, no clusters or single infections were reported in prisons. Seroprevalence among prison staff did not differ from among the general population (4,8% and 3.3% versus 6,9%). (48) (49).

HBV and travellers

Of reported HBV cases, 16% is acquired outside The Netherlands by travellers visiting areas of intermediate and high endemicity. Half of these infections are acquired through sexual exposure (39). Especially male, long term travellers are at risk, either through sexual exposure or working in health care (50).

HBV of unknown source

During a two year enhanced surveillance project co-ordinated by the RIVM, that focused on tracing the source for all MHS reported patients nationwide, a probable source could not be identified in 20% of cases (39). In Amsterdam, over the last decade, this percentage is likewise 20 (41). (Figure 4).

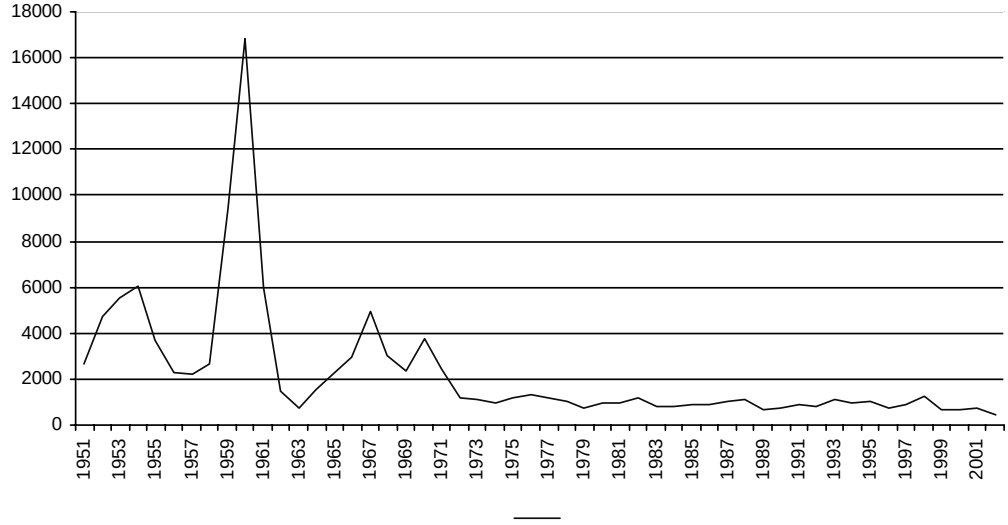
HAV notifications

Since 1951, cases of HAV are notifiable, being reported as “infectious hepatitis”. Since the last epidemic occurred in 1960, nationwide incidence has decreased to an average of annually 900 patients. In the last four years, MHS notifications were 700 or less, equalling an incidence of 2.7-4.4/100.000. The Amsterdam notifications likewise show a decrease after 1998, being 20-29/100.000 before and 7-14/100.000 after that year.

The notifications show no cyclic pattern (Figure 2HAV), but a seasonal pattern is pronounced, representing children with parental origin in HAV-endemic areas who return from summer holidays in the country of origin. They bring HAV infection, sometimes followed by a second and third seasonal incidence peak, caused by secondary and tertiary cases (51; 52).

Figure 2HAV

Annual HAV notifications to the MHS in the Netherlands, 1951-2002.

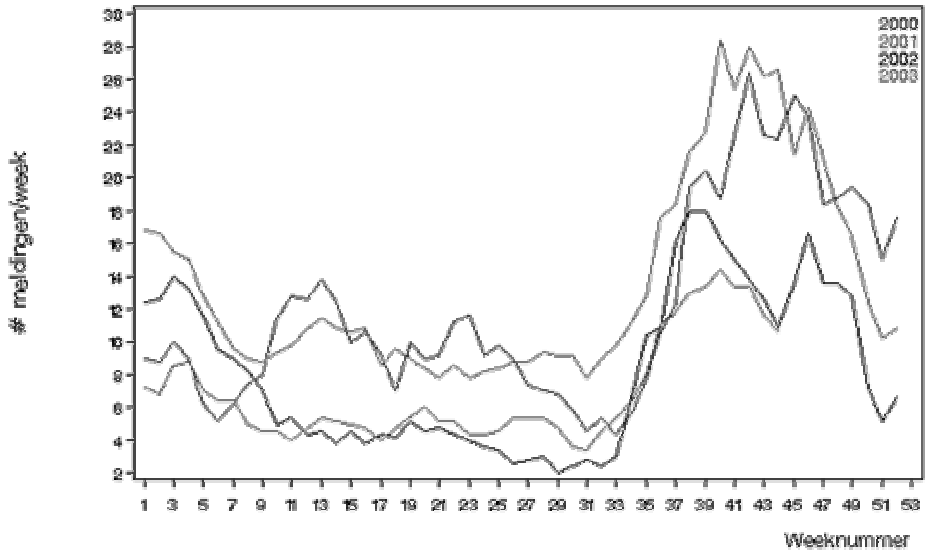


source: IGZ/RIVM

Figure 3 Seasonal variation of viral hepatitis B and A in The Netherlands

Figure 3HAV

Seasonal patterns, HAV based on number of MHS reports per week (#meldingen/week) to the Health Inspectorate for the years 2000-2003



source: www.rivm.nl/isis, accessed November 17, 2003

There is considerable underreporting (53). The Amsterdam Sentinel Project in 1979, estimated that physicians report 58% of acute symptomatic HAV (54). For the years 1994/95, the National General Practitioners' Sentinel project (CMR/NIVEL) analyzed that less than 45% of cases were reported to the MHS. But, unexpectedly, in the years 1996/97, the incidence of national notifications was higher compared to sentinel estimates (22). With capture-recapture analysis, the RIVM estimated that in the late 1990s only 27% of notifiable HAV cases are reported (20).

Serological surveys

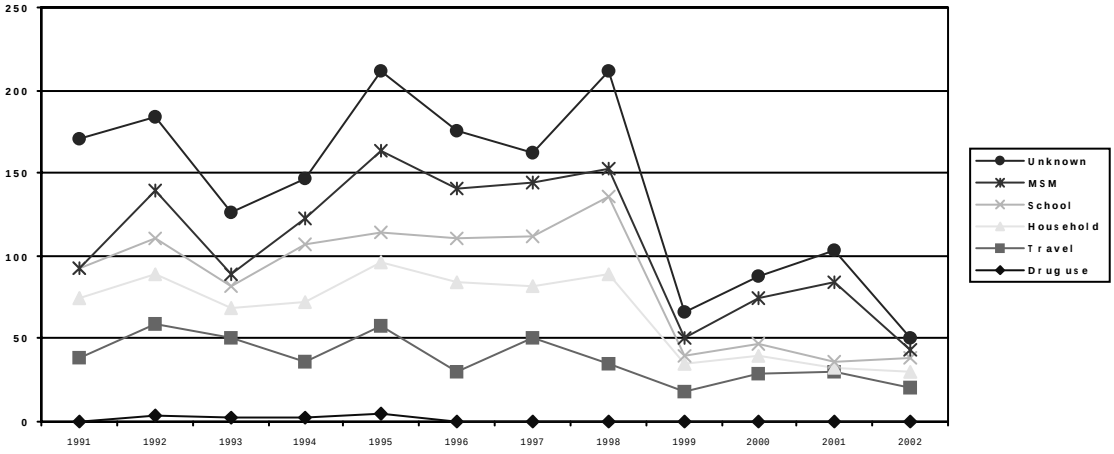
As everywhere in the world, HAV seroprevalence increases with age (55). Sera of 7367 Dutch residents, a representative sample of the population, showed a seroprevalence below 10% for persons born after 1960, and 77% for persons born before 1945 (56).

HAV epidemic profile

Seasonal increases can, in certain years, lead to protracted transmission lasting several months. With increasing numbers of susceptibles, multiple introductions can lead to overlapping outbreaks and community wide upsurges (57). For unknown reasons, 1998 was such a year.

Figure 4HAV

Annual notifications to the MHS of acute symptomatic HAV in Amsterdam, 1991-2002. Source: annual reports Dept. Infectious Diseases, MHS Amsterdam



HAV specific risk groups

Most patients are infected through household contacts with a known HAV patient (24%) (58). In Amsterdam this group consists of 20% of cases (Figure 4). Since the early 1980s, transmission has been noted among MSM in Amsterdam (59), and elsewhere (60; 61). In the Amsterdam notification data, MSM contributed 12% of cases until the mid-1990s and 20% since then (Figure 4). The mechanism responsible for this increase might be the change to sexual techniques that are regarded as “safe” with regard to blood-borne pathogens (HIV, HBV), but favour pathogens with faecal-oral transmission (HAV, Shigella). New MSM continue to come to Amsterdam, of whom the majority is susceptible.

Contrary to other low-endemicity areas, drug users (DU) are not an important risk group for new HAV infections in Amsterdam (62-65). DU cases were reported annually up to 1995, but since that year, none have been confirmed in this group (Figure 4HAV).

HAV occupational risks

Data on occupationally acquired HAV infections are limited. The Netherlands Centre for Occupational Diseases (NCVB) recorded 5 such cases in more than six years: 2 employees in primary education, 2 in aviation, and one in sports and recreation. The Utrecht MHS investigated HAV seroprevalence in 232 primary-school teachers of Dutch origin and found that, despite 45% of pupils being of foreign origin, prevalence of antibodies to HAV (antiHAV) in teachers compared to the general population (29%). However, controlling for the teachers’ age, sex, travel history, and having children in their house, investigators found that teaching groups with more than 50% children from countries of high endemicity, was an independent risk factor for antiHAV, with an odds ratio 2.9 (95% confidence interval 1.2-6.8) (66). Analysis of enhanced surveillance and notification data in the four largest cities of this country (52) suggests secondary spread, but offer no information on occupational hazards. Like teachers, healthcare workers are exposed to children but, again, no Dutch data is available, and no seroprevalence studies have been done. There is a theoretical risk that sewer workers could inhale aerosols containing HAV (or other pathogens), but international data are conflicting and unavailable from the Netherlands.

HAV and travellers

In the national registry of reported infectious diseases, 6% of notified HAV cases in adults relate to travel. In Amsterdam, 26% relate to travel. HAV poses an occupational risk for people whose work takes them to countries of high endemicity often and/or for extended periods.

HAV of unknown source
In the national registry, 8.6% of adults had an unknown source (20). In MHS Amsterdam, for both children and adults, no source could be found in 24% of cases.

Control of viral hepatitis B and A in the Netherlands

Both HBV and HAV are vaccine-preventable, but universal vaccination appears to have unfavourable cost-effectiveness ratios compared to other preventive interventions in The Netherlands (15; 20). Regarding HBV, a vaccine is on the market since 1982, plus two decades of discussions and policy making. The HAV-vaccine is so recently available, that a strategy is not yet on the public health agenda in the Netherlands, while other European areas (Catalunya, Spain; the Puglia, Italy) have successfully introduced universal vaccination with a combined HBV/HAV vaccine.

HBV hygienic precautions
As a parenterally transmitted pathogen, HBV is especially controlled in healthcare setting, by avoiding contamination of instruments, universal precautions for invasive procedures (ideally used also in tattoo, piercing, and nail studios), screening of blood products, tissue and organs and, equally important, educating healthcare workers and the public. In healthcare, single use of needles goes without saying, and for IDUs harm reduction programs, including needle exchange programs, are in place.

HBV notification and post-exposure prophylaxis
Even now, in the 21st century, notification remains a cornerstone in HBV control. For all reported cases, serological markers are evaluated and patients are interviewed by MHS nurses, who try to trace contacts, identify sources, and protect susceptible contacts through vaccination. Needle sticks and other percutaneous and permucosal accidents (as well as sexual) are to be reported according to MHS procedures or, in institutional setting, according to internal procedures. Immediate evaluation and post exposure prophylaxis is provided if justified (67). In a recent review of the literature (68), only using hepatitis B immune globulin (HBIG) was regarded sufficiently effective to continue its use in the Netherlands (42). Combining HBIG with vaccination is advised only, if high-risk accidents are likely to recur.

HBV vaccination

TARGETED GROUPS

The Dutch HBV vaccination policy has been targeted at groups at high risk for acquiring the infection. After the Health Council gave its first advise in 1983, only two of its strategies were implemented in that decade: one for medical risk groups and one for pregnant women and their babies (69). For this neonatal post exposure immunization two different approaches were implemented. In both programs, the obstetrical care provider administers HBIG. Nationally, for newborns of chronically infected mothers, HBV vaccine is added to the universal childhood vaccination program (RVP) and vaccine is administered, with the childhood vaccinations (diphtheria, tetanus, pertussis, poliomyelitis; DTKP) at months 2, 3, 4 and 11. In Amsterdam, antenatal screening and neonatal vaccination were made part of the MHS infectious disease control activities. There, newborns of HBV carrier mothers receive HBV vaccine at months 0, 1 and 6. For other identified risk groups, no programs were implemented. In 1996 these strategies were to be evaluated, but data were lacking (17). The Council again repeated its advise of 1983, and noted that the government had not made HBV vaccine available for behavioural risk groups. As for vaccination of household contacts, the Council noted that a regulation was in place, making such vaccinations possible, but no evaluation had been done. Five years later, in 2001, the Council advised against universal vaccination at that time (31). In 2003, it advised an additional target group for HBV vaccination: newborn children with origin in countries of intermediate or high endemicity (27). The Council's advice was implemented at various speeds and at present, practically all risk groups are addressed in the national policy (70).

OCCUPATIONAL RISK GROUPS

By European regulation of 1994, employers are obliged to protect with vaccination employees at increased risk for acquiring HBV. In 1998, the Dutch Minister of Health pleaded for active implementation of this regulation. It needed a HBV outbreak in 1998, related to a chronically infected surgeon (34), to make hospital administrators and government regulators truly aware of the obligation to vaccinate. In 1999, the European regulation was integrated into Dutch policy rules (71). Yet, healthcare workers still seem undervaccinated (72; 73). MHS facilities try to improve vaccination coverage of such workers in their area, but it remains the responsibility of each healthcare employer. Many non-healthcare occupational risk groups fall under the European regulation, but it is unknown if their employers have implemented protective vaccination for all relevant employees.

TRAVELLERS

Travellers to areas of intermediate or high endemicity are advised to have HBV vaccination for travel exceeding 3 months or for travel of shorter travel if additional risk factors are suspected (occupational, sexual or other). The vaccination cost is paid for by the travellers, their health insurance, or their employer.

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Based on mathematical modelling studies by the RIVM (15), the Health Council concluded that universal vaccination against HBV does not compare favourably with other preventive interventions, from the financial point of view. However, the Council offered additional remarks on some assumptions. A second calculation included the effect of horizontal transmission within households and brought similar conclusions. Universal vaccination might reduce the incidence of acute symptomatic HBV, but will not reduce the number of chronic carriers, who weigh heavily on the cost side of the equation.

HAV hygienic precautions

Personal hygiene and sanitation (provision of safe drinking water and proper sewage disposal) are the mainstay of HAV control. However, the Netherlands has no educational programmes directed specifically at prevention of HAV. Quality control for the food industry is being left to the producers, with limited inspection by the national food authority. A hygienic code was developed by various branches of industry and the professions, as well as one for hygienic precautions at home.

HAV notification and post-exposure prophylaxis

The rationale for MHS notification is to enable rapid response in order to prevent new cases: find a source, stop transmission (with hygienic precautions), and protect susceptibles (with immune globulin). The guidelines for post-exposure prophylaxis (74) are in transition due to new data on the possible rapid protective efficacy of vaccination and safety questions regarding the use of blood and blood products as sources of immune globulin (75). At the start of our studies it was unknown if the present secondary prevention policy is effective and if it prevents further transmission.

HAV vaccination

TARGETED GROUPS

Nationally, one risk group (haemophiliacs) gets HAV vaccine reimbursed by formal regulation. No other national HAV vaccination plan is offered, even for pa-

tients with chronic liver disease (76).

OCCCUPATIONAL RISK GROUPS

Several occupations are linked with increased risk for acquiring HAV and fall under the European regulation on protection of workers against biological agents. The list of agents concerned includes HAV (and HBV). In the Netherlands, this protection is the responsibility of employers, advised by their occupational health service. They are expected to vaccinate their workers, but their response to this policy is never evaluated. There is also no specific vaccination policy to cover workers in day care, primary schools, nursing staff of paediatric wards, or sewage workers. A fatal case of fulminant hepatitis in a primary school teacher in 1998 did not push the regulatory bodies to implement the relevant guidelines.

TRAVELLERS

In the private travel industry, vaccination is recommended for all travellers to areas of intermediate or high HAV endemicity, regardless of the duration of the stay. This applies also children of parents originating from countries of intermediate or high endemicity, but with no national policy or regulation, MHS facilities and Health Insurance companies have implemented local policies. For example, on vaccination days in early summer, the HAV vaccine is offered for a reduced price or free of charge. The coverage of these initiatives is not nationally evaluated, but in the Amsterdam evaluation the coverage averaged only 60%.

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There is no public or government discussion on HAV prevention, although the Ministry requested the RIVM to perform a desktop study on the costs and benefits of introducing universal vaccination. The outcome of this preliminary analysis showed universal vaccination not to be within the cost-effectiveness limits that are acceptable for preventive interventions (20).

Virological typing

Descriptive epidemiology fails to present a complete picture of HBV/HAV epidemiology and consequent control strategies. Additional information might be gathered from viral epidemiological data. With modern techniques, isolates of viruses can be (geno)typed in detail. Strains cluster in groups and sometimes transmission can be followed up by detailed typing of a single strain. HBV and HAV are unrelated viruses, that have in common only the tropism for the hepatocyte. Typing for each virus is classified differently. Combining information on the strain of a

virus with information on the patient in whose excreta or bodily fluids the virus was isolated is called molecular epidemiology. At the start of our studies, no molecular epidemiological data on HBV/HAV were available for the Netherlands.

HBV

The ubiquitous HBV virus is the smallest known DNA virus, a 42 nm sphere, with a genome of 3200 base pairs, in a partially double stranded DNA (77). It has features in common with retroviruses, to which it may be related (78). It is an efficient virus, since the genome encodes overlapping open reading frames, allowing 63% of its genome to be translated in more than one reading frame. Even a one-point mutation in the gene may affect both structure and function of the virus, reducing infectiousness or viability. As a result, the virus is rather stable. There are four open reading frames: preS/S (surface), X and P (polymerase), and preC/C (core) gene. The S open reading frame encodes three proteins that are large, medium-sized, and small, comprising the surface antigen (HBsAg). Replication occurs through production of an RNA copy, which is then converted back to DNA by use of its own reverse transcriptase (DNA polymerase). The polymerase serves also as a reverse-transcriptase, since replication requires RNA intermediates. HBV core antigen is the nucleocapsid that encloses the viral DNA. When HBcAg-derived peptides are expressed on the surface of hepatocytes, they induce a cellular immune response that leads to its killing (79). HBV "e" antigen (HBe), a cleavage from the core gene and then modified and exported from liver cells, serves as a marker of active replication.

The serologic heterogeneity of the HBsAg was documented in the 1970s (80; 81) by finding one common antigenic determinant (*a*) and two pairs of mutually exclusive determinants, *d/y* and *w/r*. It is now generally accepted that there are, in further detail, at least nine different HBV serotypes (called subtypes), which makes the subdivision rather complicated: *ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq*$^{+}$*, adrq*$^{-}$. (82). Starting with the complete HBV-DNA (83; 84), sequencing of the complete S gene (85) and later limited sequencing within the S gene (86), showed that the S gene can be typed in seven genomic groups or genotypes, designated A – H. The cited genetic virological studies have generally analyzed strains originating from individuals with chronic HBV infection (85; 87-91), thus reflecting the epidemiological situation of vertical transmission in the areas in which they dominate. The typing is further refined by splitting certain genotypes in subgroups (92), or clades (86). There is, to a certain extent, accordance between genetic and serological classifications (90; 93), but as more sequence data come available, further divergence is anticipated. The geographical distribution of serotypes and genotypes is indicated in Figure 1HBV.

The genotype F (corresponding with serotype *adw4*) shows the most intratypical variety (94), which suggests that genotype F may be the origin of all HBV viruses.
Experience with viral genotyping for public health purposes shows that sequencing can link cases in an outbreak setting (33; 95-98) and assist in tracing their origins, as in an HBV outbreak among drug users in Scandinavia (99). Also, genotyping relates certain genotypes to certain risk groups (100).

HAV
The ubiquitous HAV virus is an extremely small non-enveloped (naked) 27-32 nm RNA virus of symmetrical icosahedral structure; it is morphologically indistinguishable from other *Picornaviridae*, to which family it belongs. The virus has a single-stranded RNA, of 7478 nucleotides, that expresses three proteins (VP1, VP2, VP3) but no envelope protein. Its genetic difference from other *Picornaviridae*, and its being the only to cause hepatitis, are reason to establish its own genus, *Hepatovirus*.
There is only one known serotype, but, based on sequence variability, seven genotypes exist, I – VII, that differ 15-25% in a small region spanning VP1/2A (101). Genotypes I, II, III and VIII infect humans, whereas IV, V, VI are found in primates that are kept in captivity (102). There seems to be a geographical distribution of genotypes, but it should be interpreted with caution; genotyping is performed on a small part of the gene, and studies to date are on small and often selected populations (103). The apparent geographical distribution of genotypes is described in Figure 1HAV.
There can be much value in HAV genotyping for public health, as seen when sequencing linked two separate outbreaks later confirmed to be caused by one source of contaminated strawberries (104). Molecular data have added value in many an outbreak investigation, either in confirming suspected strain relations (65; 105-108), or conversely, in ascertaining retrospectively that several strains were involved in seemingly uniform outbreaks (110-112). As with HBV, genotyping of HAV helped relate an outbreak to drug users in Scandinavia (64).

Aims of the studies in this thesis

HBV
In the Netherlands, a targeted approach is the choice to control HBV. The first and most important target group is new born children of HBV carrier mothers for which two different approaches exist. Always, HBV immune globulin is given immediately at birth by the obstetrical care provider. In the national program, an

adult dose of HBV vaccine was given to neonates of carrier mothers, at the regular visits for the universal childhood vaccination program, in addition to diphtheria, poliomyelitis, pertussis and tetanus vaccination, at month 2, 3, 4 and 11, until March 2003, when it changed to a child's dose.
In Amsterdam, the adult dose HBV vaccine is administered by public health nurses of the MHS of the Department of Infectious Diseases. Dosing schedule is month 0, 1 and 6. The first dose is given in the first week of life. If the mother is HBe antigen positive, the first dose is administered within 48 hours after birth. It is unknown how effective these two different programs are, since neither has been evaluated since the early 1990s. CHAPTER 2 describes our evaluation of the Amsterdam approach to see if the program is effective and how it compares to similar programs elsewhere in the world.

Another high-risk group for acquiring HBV is household contacts of carrier individuals. In Amsterdam, such contacts of chronically infected women that are identified through antenatal screening, are targeted through the neonatal vaccination program. This relatively new aspect of the Amsterdam approach has not yet been evaluated. Meanwhile, tracing contacts is not only relevant for carrier women, but should be performed for all chronically infected individuals. By law, all carrier individuals in the Netherlands have to be reported to the local MHSs since April 1999. Data of the integrated household tracing program are useful for all MHS facilities in the Netherlands. CHAPTER 3 describes our evaluation of this program of coverage and the compliance of susceptibles with the vaccination series.

In addition to these targeted groups, those at high-risk because of sexual behaviour or drug use should be vaccinated, according to the 1983 advice of the Health Council, but no specific funding or program has materialised. Since MHS facilities were regarded as the most appropriate coordinators for such a program, our pilot program was instituted of recruiting these risk groups with MHS outreach. We coordinated the pilot of seven MHS facilities, including Amsterdam, and CHAPTER 4 evaluated how the various risk groups were covered at this sites.

It remained uncertain whether all the targeted strategies combined would actually suffice to stop, or substantially reduce, HBV transmission or otherwise affect the HBV epidemiology among persons in the Netherlands outside the targeted high-risk groups. Since data on patients failed to give a detailed picture of the HBV epidemiology, we investigated the molecular epidemiology for Amsterdam in the period 1992-1997. CHAPTER 5 presents findings that add essential information on the true transmission routes in this area and might have relevance for other

areas in the country, since transmission routes follow similar patterns in similar risk groups.

HAV

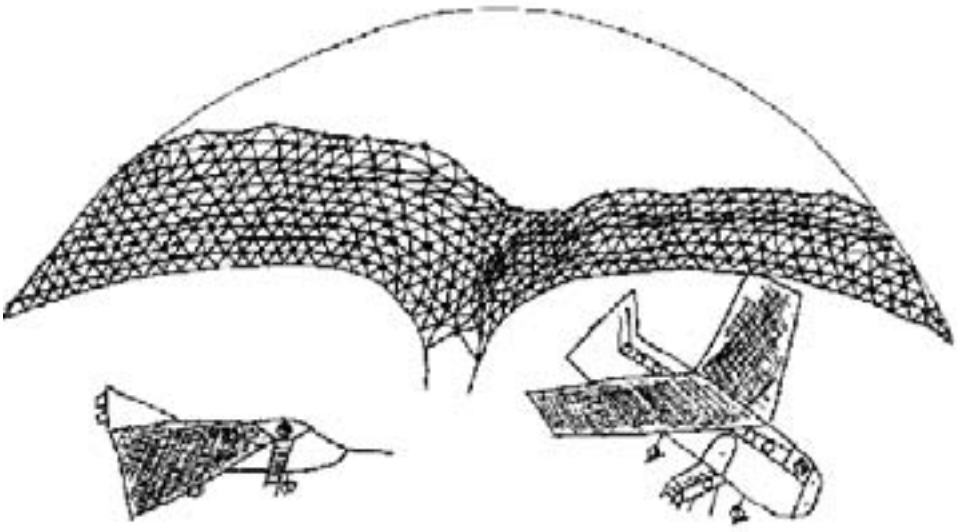
It is unknown if the present HAV prevention policy of MHS notification, followed by rapid post-exposure prophylaxis, has sufficient impact. We know that there is considerable underreporting of HAV with asymptomatic and undiagnosed infections. This factor combined with incomplete coverage of HAV traveller vaccination, in particular the children visiting their country of origin, raises doubts about current HAV control strategy. Descriptive epidemiologal data showed a decrease of notifications since 1998, but how and how often the virus is still introduced and if it is further transmitted after importation is unknown and needs clarification. CHAPTER 6 addresses the policy of reporting to the MHS, by evaluating control and outcome in contacts of reported cases.

The large number of new cases with unknown probable source might suggest continuous low level transmission. However, it might also be explained by multiple asymptomatic undetected introductions that would be reduced with enhancement of the vaccination strategy. In fact, it was completely unknown if local transmission occurs, and equally unknown if the existing incidental cases with unknown source are possibly food-borne or can be related to secondary or tertiary (silent) transmission originating from import cases. The answers on these questions are essential for deciding on the optimal prevention strategy. CHAPTER 7 describes how molecular technique was introduced and implemented.

The HAV vaccination coverage among Dutch travellers is unknown, as is its coverage among children visiting their country of origin. The MHS-notified cases are a minority of infections, and many notified cases can not be traced to a probable source. Occasional outbreaks in the general public are reputed to be related to import cases, but never confirmed; occasional outbreaks among MSM, are likewise linked to import cases without confirmation. Such outbreaks might equally be endemic in a small subgroup of the population. CHAPTER 8 presents the results of two years of specimen and data collection and, in combining these data, we are able to give new information on HAV epidemiology.

Conclusion

Both illnesses HBV and HAV cause significant morbidity, even in this country of low endemicity. They lead to a few hundred hospital admissions and approximately 60 deaths annually, causing direct and indirect costs associated with hospital expenses and days lost from work by adults who are ill or must care for a sick child. For both illnesses, universal vaccination seems not justifiable on economic grounds, but the disease burden may well be larger than currently believed. The source can not be found in 1 out of every 5 reported cases, leading to uncertainty on the predominant transmission routes and the adequacy of prevailing control strategy. The studies presented in this thesis, seek to contribute to better understanding of HBV/HAV epidemiology and the consequent control strategies.



Hepatitis A virus is non-enveloped of symmetrical icosahedral structure

The striking building of the Aviation Museum (Aviodome) at Schiphol, stemming from 1971, will be demolished. The characteristic dome has been many years the largest aluminium dome in the world. The building was designed by the architect Richard Buckminster Fuller, with a geodesic dome. This structure is comprised of a complex network of triangles that form a roughly spherical surface. The more complex the network of triangles, the more closely the dome approximates the shape of a true sphere. By using triangles of various sizes, a sphere can be symmetrically divided by thirty-one great circles. A great circle is the largest circle that can be drawn around a sphere, like the lines of latitude around the earth, or the equator. Each of these lines divide the sphere into two halves, hence the term geodesic, which is from the Latin meaning "earth dividing". The dome is a structure with the highest ratio of enclosed area to external surface area, and in which all structural members are equal contributors to the whole. There are many sizes of triangles in a geodesic, depending on the frequency of subdivision of the underlying spherical polyhedron.

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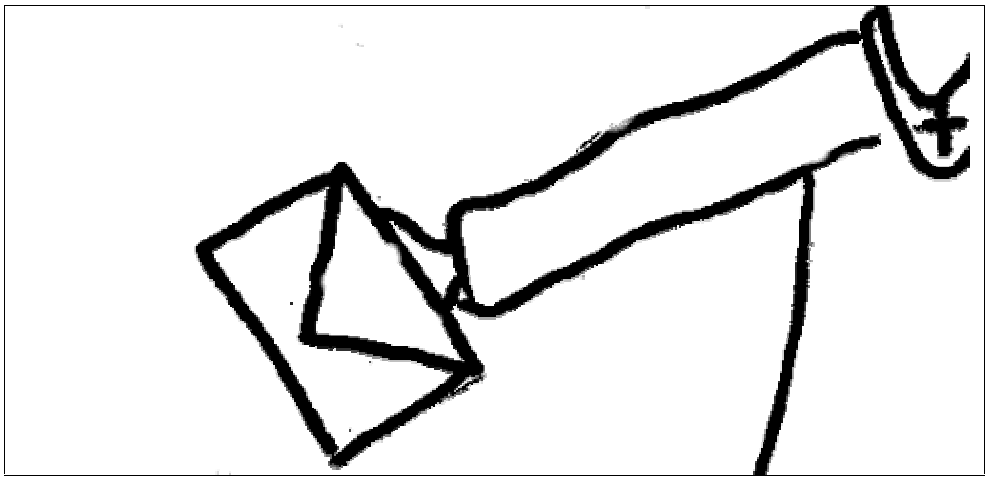
Chapter 2

Evaluation of the hepatitis B antenatal screening and neonatal immunization program in Amsterdam, 1993-98

Jim E. van Steenbergen (1,2), Anne Leentvaar-Kuijpers (1), Dorothé Baayen (1), Nicole H.T.M. Dukers (1), Gerard J.J. van Doornum (3), Anneke van den Hoek (1), Roel A. Coutinho (1,4)



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1. GG&GD (Municipal Health Service), Dept. of Infectious Diseases, Amsterdam, The Netherlands (NL)
2. LCI (National Coordination Communicable Disease Control), Utrecht, NL
3. GG&GD, Public Health Laboratory, Amsterdam, NL
4. Academic Medical Center, University of Amsterdam, Dept of Human Retrovirology, Amsterdam, NL

Abstract

Aims- To evaluate the enhanced antenatal hepatitis B screening and neonatal immunization program in Amsterdam.

Method- Retrospective analysis of routinely collected data on serological screening of expectant mothers for hepatitis B surface antigen (HBsAg), on timing of administration of immune globulin and vaccinations to the neonate, and on postvaccination serological evaluation of the antibodies to hepatitis B surface antigen (antiHBs) in the new born children.

Results- In the period 1993-98 there were 691 HBsAg positive expectant mothers reported. The coverage of the screening was calculated at 97%. HBsAg-prevalence was high in women from Ghana and South-East Asia, and lowest in Dutch women. Hepatitis B immune globulin (HBIg) was administered within 24 hours to 95,9% of the neonates; 99,7% completed the vaccination series. About 6 weeks after the third vaccination the titer antiHBs was $\geq$ 100 IU/L in 85% of children; in 12% the titers were 10-100 IU/L; 3% had titers < 10 IU/L, of whom 3/521 initally had HBsAg. Low birth weight (OR 3.77), being a boy (OR 1.64) and country of origin were predictors of low postvaccination titers.

Conclusions- Coordinated by 0,5 full time equivalent (fte) additional staff, the program was relatively cheap and successful.

Introduction

Since November 1989, all pregnant women in the Netherlands are screened for hepatitis B surface antigen (HBsAg) at their first visit to the obstetrician, midwife or general practitioner [1]. The obstetrical care provider administers hepatitis B immune globulin (HBIg) immediately after birth, to neonates of HBsAg-positive mothers. The HBvaccine is administered in the Child Health Clinic at the time of the usual childhood vaccinations, usually at month 3, 4,5 (month 2,3,4 since 1999) and 11.
In the city of Amsterdam with 720.000 inhabitants and approximately 10.000 annual births, we designed and implemented in 1989 a centralised and enhanced screening and immunisation program. All HBsAg-positive laboratory results of antenatal screening are reported to the Municipal Health Service (MHS). The MHS organises confirmatory tests, supervises the provision of HBIg, and organises hepatitis B vaccination of children. The Amsterdam vaccination schedule is at month 0, 1 and 6, with a postvaccination screening at month 7. Here we present the results of the Amsterdam screening program, the differences in prevalence according to country of origin, and the factors related to low postvaccination titres.

Methods

Screening procedures
At their first antenatal care visit, scheduled in week 14 of gestation, all pregnant women in Amsterdam are tested, with informed consent, for hepatitis B surface antigen (HBsAg). The Public Health Laboratory (PHL) receives most of blood samples, a minority is sent to other laboratories in town. The laboratories report HBsAg positive results to the MHS. In a letter, the MHS invites the expectant mother for confirmatory testing for HBsAg (Abbott Auszyme 1993-1996, Abbott Laboratories, North Chicago, Il.,USA; Abbott AxSYM MEIA 1997-98, Abbott Diagnostics, Wiesbaden, Germany). If the result is HBsAg-positive the complete serological status is determined: antibody to core antigen (antiHBc), e antigen, antibody to e antigen (HBeAg and antiHBe) using Abbott tests, EIA 1993-94, IMx 1995-96, AxSYM MEIA 1997-1998. Mothers are regarded HBsAg positive if HBsAg has been confirmed by the PHL in the second sample.
In a visit with a public health nurse (PHN) at the MHS, the expected date of delivery is registered in the computerised file, and the PHN arranges for a supply of HBIg to be available at the expected place and date of delivery.

Intervention

As soon as possible after the delivery (always within 48 hours), the midwife or physician administers HBIg to the new-born and informs the MHS about the date and time of HBIg administration. Within a week, the PHN verifies that HBIg was given and arranges for the first dose of hepatitis B vaccine (HBV-I, Engerix™-B, SmithKline Beecham Biologicals, Rixensart, Belgium) to be administered at home or in the hospital. For the second dose (month 1, HBV-II) and third dose (month 6, HBV-III), the mother and baby visit the MHS.

Serological postvaccination evaluation

Six weeks after the third vaccination, blood is taken for testing of the antiHBs response by the PHL. If the antiHBs titre is over 100 IU/L, the child is discharged from follow-up. Children with initial antiHBs titre of 50-100 IU/L receive one re-vaccination without retesting of antiHBs; those with initial antiHBs titre of 10-50 IU/L receive two revaccinations with retesting of anti-HBs. In children with antiHBs titer of<10 IU/L, the complete serological status is determined. If the child is HBsAg positive, it is tested annually and, if justified, referred for further care and antiviral treatment. For the co-ordination of the program, the case-management and tracking a 0,5 full time equivalent PHN is appointed to the program. Home visits and consultations are done by regular communicable disease control staff (PHNs) at MHS.

Statistical methods

The number of pregnancies at 14 weeks gestation is higher than the number of live and still births. To calculate the number of pregnancies in Amsterdam we use the percentage of pregnancy loss of the HBsAg positive women in our register: 691 pregnancies leading to 656 births, i.e. a loss of 5%. To calculate the coverage of the screening by both the PHL and other laboratories, we assumed equal HBsAg prevalence in both populations screened. The number of live and still births is obtained from the Municipal birth registry. Country of origin is determined by the origin of either parent: if one of the parents is of non-Dutch birth or non-Dutch nationality, the child is regarded as belonging to that parent's country of origin. For the calculation of HBsAg prevalence by country of origin we used live births as denominators.

In Amsterdam, the MHS data are entered in a local database and for this study we transcribed the relevant data to a DbaseIV file. To evaluate trends or differences in prevalence, we used a Poisson distribution [2]. In calculating the risk factors for low post-vaccination titres, we included all univariate statistically significant variables in a multivariate analysis, using a stepwise backward procedure [3]. We tested for interactions among variables included in the multivariate model

and examined the confounding effect of other variables. Logistic regression was used to obtain univariate and multivariate odds ratio's (ORs) and 95 percent confidence intervals (CIs) were used to quantify variation in estimates.
In general, confounding was considered to occur when inclusion of a variable (or combination of variables) in the multivariate model resulted in a change of more than 15 percent in the ORs of the factors that were already present in the model. A p-value of less than 0.05 was considered statistically significant.

Results

Screening
From 1993 to 1998 in Amsterdam, 57.889 births were recorded, including 56.756 live and 1.133 still births. In this period 51.457 sera (86%) were initially screened by the Public Health Laboratory (PHL). The calculated number of pregnancies was 59.784. The coverage of the antenatal screening program was 97%. We found 691 HBsAg-positive samples, of which 612 were reported by the PHL. The prevalence of HBsAg by country of origin ranges from 0% in women from the Netherlands Antilles to 8,9% in women from Ghana. Women from Southeast Asia had a HBsAg-prevalence of 7,6%; Turkish women 3,6%; women from the two largest non-European groups in Amsterdam (Morocco and Surinam) had 1,5% and 1,7% respectively. Native Dutch women, had 0,07%, of whom 11/19 had a known risk factor. Of HBsAg positive sera 61/688 were positive for the hepatitis B e antigen (HBe/HBsAg-ratio 8,9%); this ratio was highest in women from South East Asia (26%). In 15/688 HBeAg and antibodies to the e antigen (antiHBe) were negative.

Passive immunisation
Of 691 HBsAg-positive pregnant mothers, 9 delivered twins and 44 did not deliver a live child, resulting in 656 newborns who were eligible for HBIg. Of these, 629 (95,9%) received HBIg within 24 hours, 16 (2,4%) received it between 24 and 48 hours, and 8 (1,2%) received it more than 48 hours after delivery but within the first week. Three newborns (0,5%) did not receive HBIg but received HBV-I in the first week.

Table 1

HBsAg prevalence in 56.756 sera of mothers giving life birth by country of origin[1)], 1993-98

Ethnic origin	Number live births[3)]	Number HBsAg+	% HBsAg+[2)]
The Netherlands	25819	19	0,07
Moroc	7337	113	1,54
Surinam	6522	111	1,70
Turkey	4411	157	3,56
Ghana	1473	131	8,89
Nl Antilles/Aruba	991	0	0,00
SE Asia	925	70	7,57
Other	9278	90	0,97
Total	**56756**	**691**	**1,22[3)]**

1) As determined by nationality or country of birth of mother or father or child considering any non-Dutch as dominant
2) Prevalence in all groups differs statistically significant (p< 0,01) from the remaining groups.
3) Excluding stillbirth in the denominator, leading to overestimation of the HBsAg prevalence.

Active immunisation

Four newborns were not eligible for HBV-I because one died, two were transferred to the national program when they moved outside Amsterdam, and one was not yet scheduled at the time of this analysis. Of the remaining 652 children, 475 received HBV-I in the first week of life (73%), 136 in week two (21%), and 41 after week two (6%).

For the second vaccination (HBV-II), eight children were not eligible because one died, one was transferred to the national program, and six were not yet scheduled. Of the remaining 644 children 508 received HBV-II in four to six weeks after HBV-I (79%), 108 children six to ten weeks after HBV-I (17%), and in 28 cases more than 10 weeks after HBV-I (4%).

For the third dose (HBV-III), 50 children were not eligible because two died, six were transferred to the national program, and 42 were not yet scheduled. Of the remaining 594 children, HBV-III was given on time, i.e. within 7 months after HBV-II, to 540 (91%), and late to 52 children (9%). Two children were lost to follow-up.

Postvaccination screening

At the time of this analysis, 521 children of the 547 eligible to be tested had come for the determination of a post-vaccination titre (95%). Of the 521 children 440 (85,5%) were high responders to the vaccine, with an antiHBs titre ≥ 100 IU/L, 64 (12,2%) were low responders, with a titre 10-100 IU/L, and 17 (3,3%) were non-responders to the vaccine, with a titre <10 IU/L. Three non-responders were HBsAg positive. Of these, one lost HBsAg spontaneously after two years, one seroconverted to antiHBs in his fifth year of life. One of the three mothers was HBeAg-positive.

Revaccinations

Excluding the three HBsAg positive children, revaccination was performed according schedule for all 78 neonates with titres <100 IU/L. Of these, 31 with antiHBs titres 50-100 IU/L received a fourth vaccination (HBV-IV) without reanalysis of an antiHBs titre; 33 with antiHBs titres 10-50 IU/L received the fourth and fifth vaccination (HBV-IV and HBV-V); and 14, with antiHBs titre 1-10 IU/L, received three revaccinations. Of the 47 children in the last two groups, 36 came for reanalysis of antiHBs and 28 (78%) had a titre ≥ 100 IU/L, 5 (14%) had a titre 10-100 IU/L and 3 (8%) remained <10 IU/L.

Risk factors for low anti HBs-titre (Table 2)

Univariate analysis could not identify any risk factor associated specifically with antiHBs titres <10 IU/L. However a response <100 IU/L (n=82) was more often found in boys, small babies (birth weight <2500 gr.), neonates born early (<38 weeks of gestation), in case of late administration or failure to give HBIg, and in children of certain countries of origin. Multivariate analysis showed that low birth weight, male gender and the country of origin variable were independently associated with post-vaccination titres below 100 IU/L. It showed, that children of mothers of Surinam and Ghanaian origin had a substantially decreased risk for low titres (OR 0,27 and 0,28 res.). We also examined potential confounders, including HBe status of the mother and various schedules of immunisations. ORs appeared to be stable after adding these factors to the model. Furthermore all possible interaction variables between factors that we included in the multivariate model were not statistically significant.

Selecting the three groups with high-risk for low titers, includes 98% of low titers. For this selection, i.e. male gender, low birth weight and not being of Surinam or Ghanaian origin, 84% of neonates have to be screened. Limiting the selection to male gender and low birth weight includes 67% of low titres and needs 53% of all neonates to be screened.

Table 2

Riskanalysis for low titers (antiHBs<100 IU/L) in a univariate (Odd's Ratio, OR) and multivariate analysis (Adjusted Odd's Ratio, AOR) using a set of data of 521 postvaccination sera.

		Number[1)]	OR	95% CI	AOR	95% CI
Gender	Girls	257	Ref		Ref	
	Boys	261	1,66	**1,03-2,70**	**1.65**	**1.00-2.73**
Birthweight	> 2500	463	Ref		Ref	
	<2500	31	2.30	**1.02-5.19**	**3.77**	**1.54-9.20**
Duration preg	38-42 weeks	380	Ref			
	<38 weeks	49	2.18	**1.09-4.37**	Ns	
	> 42 weeks	40	1.75	0.79-3.89		
HBe mother	Neg	463	Ref			
	Pos	47	1.74	0.85-3.59	Ns	
HBIg	On time	500	Ref		Ns	
	Not or late	21	2,83	**1,11-7.25**		
HBV-I	On time	377	Ref		Ns	
	Late	143	0.60	0.34-1.08		
Ethnicity	Turkish	127	Ref		Ref	
	Ghanaian	98	0.36	**0.16- 0.84**	**0.30**	**0.12- 0.72**
	Moroccan	95	0.76	0.38- 1.55	0.74	0.36- 1.53
	Surinam	84	0.31	**0.12- 0.80**	**0.28**	**0.11- 0.72**
	South-East Asian	44	1.05	0.45- 2.46	1.06	0.45- 2.52
	Other developing	38	2.12	0.95- 4.72	2.11	0.93- 4.79
	Industrialized world	27	0.71	0.22- 2.24	0.67	0.21- 2.16
	East-European	8	1.36	0.26- 7.15	1.33	0.25- 7.08

1) not all adding up to a total of 521 due to missing data

Discussion

Coverage

In the industrialised world, antenatal screening and subsequent immunisation of neonates of HBsAg-positive mothers is an integral part of a comprehensive strategy to eliminate hepatitis B [4,5]. This is particularly the case for Amsterdam, where the prevalence of HBsAg is higher than in the rest of the Netherlands [6]. The HBsAg prevalence for the various groups in Amsterdam is in accordance

with prevalence found in the various countries of origin [7-10]. The Amsterdam program shows better results than the Dutch national program. The coverage of screening increased from 91% in the period 1989-92 [6], to 97% in the years 1993-98. National figures after 1992 are not available, but from 1989 to 1992, national screening coverage was 84% [11]. The Amsterdam coverage ranks with the best reported results of active programs elsewhere [12-16], which range from 92 to 98%. There are two assumptions made in calculating the coverage. We assumed equal HBsAg prevalence in pregnant women referred to the PHL or to other laboratories. This appears to be acceptable, since no difference could be detected between these two populations in distribution of countries of origin or the HBe-status. We assumed a 5% loss of pregnancies for the total population. This appears to be acceptable, since this percentage in the general population is lower [17]. We thus possibly overestimate the total number of pregnancies and underestimate the actual coverage.

Centralised program

The Amsterdam program is cheap and effective in comparison to other hepatitis B intervention programs [18]. The workload for the co-ordinator will further decrease as the program becomes better known to obstetrical care providers and the at-risk population. The high coverage, obtained with limited input of additional staff, make the program a good model for those areas where hepatitis B vaccination is not included in the universal childhood vaccination program. Its design may be suitable both for the UK, where local systems need to be implemented to monitor policy and practice [19], as well as in the USA, for areas with unsatisfactory screening rates or rates of fully vaccinated children [20]. Indeed, centralised case management and home visits have reportedly played a critical role in improving compliance in the US [21].

Monitoring outcome

The Amsterdam program offers the opportunity to monitor the effect of neonatal immunisation. This is especially important as the timing of the first vaccination is sub-optimal. This should preferably be given simultaneously with HBIg. However, for practical reasons, the obstetrical care providers are asked to administer HBIg only. In the initial post-vaccination screening, we found 15% sera with antiHBs <100 IU/L. Children with a titre 10-100 IU/L are regarded as protected against clinical disease and chronic infection [22,23], but since higher titers are known to give long lasting protection against infection[24], we revaccinated children with antiHBs titres <100 IU/L. As was shown in neonates of HBsAg negative mothers [25], revaccination gave good results: 74% of children with titres <50 IU/L developed a post-revaccination antiHBs titre >100 IU/L.

Despite timely appropriate immune prophylaxis, we found, as expected [26], a few HBsAg positive cases (n=3; 0,6%), of whom two lost HBsAg over the years. Others incidentally report better results [27]. In the Dutch national program, where the first vaccination is given only at month 3, recently month 2, the percentage HBsAg positive children in the is higher [28]. This seems not related to the late timing of the first vaccination [29]. We see no argument yet in changing the procedure. Although antiHBs was not present in the three HBsAg positive children, the presence of an escape mutant should be considered and paired sera of mother and child will be further investigated.
The need for post-vaccination serologic testing in fully immunised children can be questioned. We were not able to identify a set of criteria for selective post-immunisation screening to predict titers <10 IU/L or a practical selection for titers <100 IU/L. It is disputed if low birth weight is predictive of vaccine failure [30]. Our finding that 29% of the children with a birth weight <2500 gr. have titers <100 IU/L suggests that it is prudent always to test anti-HBs in children with birthweight <2500 gr.

Acknowledgements

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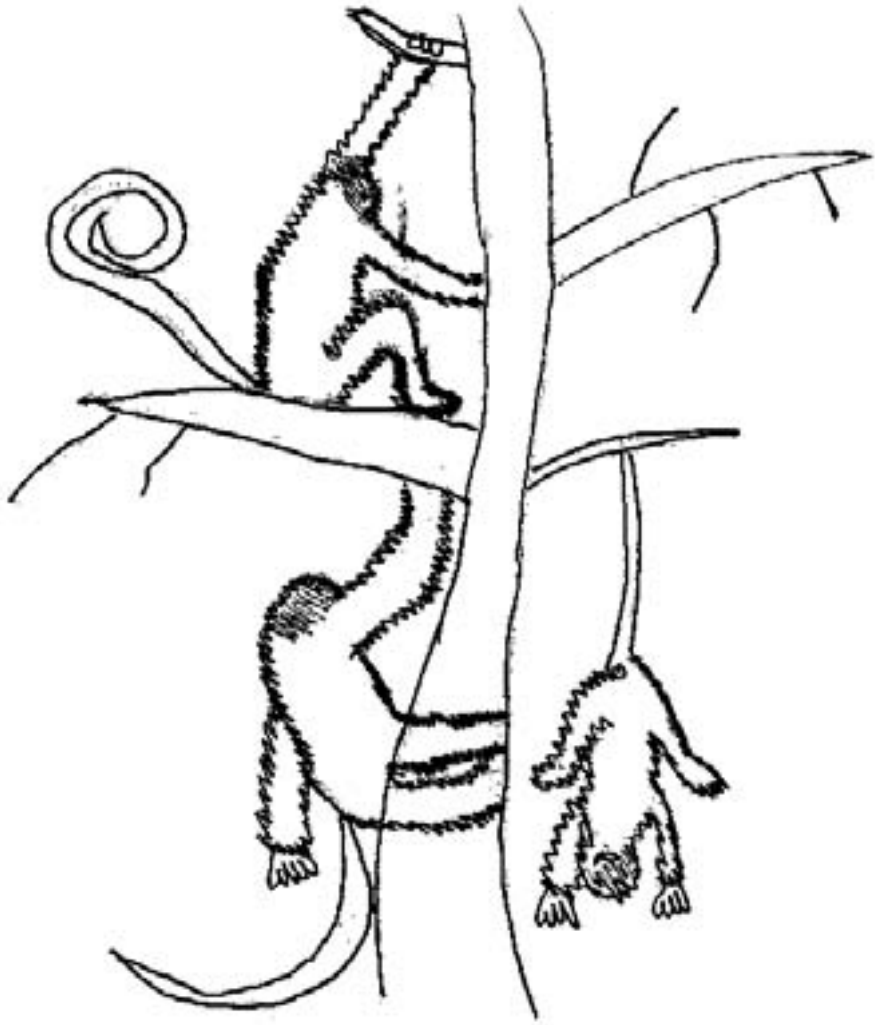

Maybe, the origin of hepatitis B lies in a species jump from primate to human. In the woolly monkey (*Lagothrix lagotricha*) a hepadnavirus was identiefied, that shows the closest relation with human genotypes of all primate hapadnaviruses. The woolly monkey is a primate of the America's.
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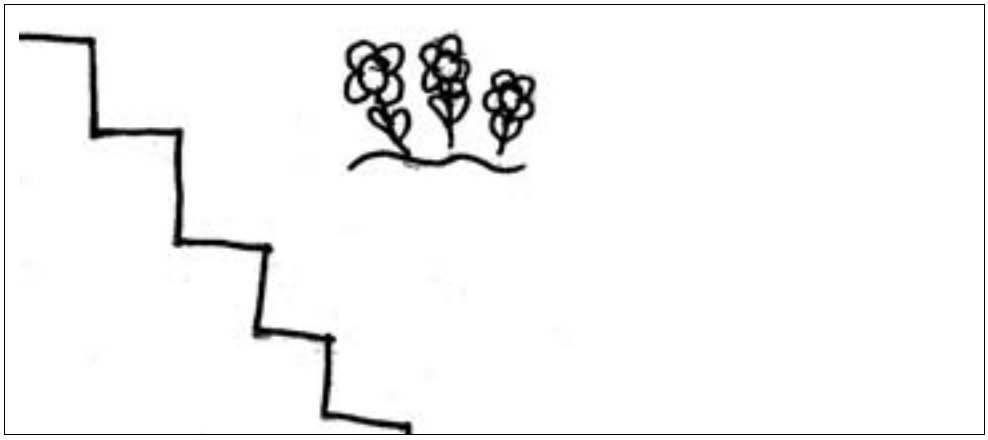
Chapter 3

Much gained by integrating contact tracing and vaccination in the Hepatitis B antenatal screening program in Amsterdam, 1992-1999

Jim E van Steenbergen(1,3) , Dorothé Baayen (1), Paul G.H. Peerbooms (2), Roel A. Coutinho (1,4), Anneke van den Hoek (1)



(Submitted)

1. GG&GD (Municipal Health Service), Dept. of Infectious Diseases, Amsterdam, The Netherlands (NL)
2. GG&GD, Public Health Laboratory, Amsterdam, NL.
3. LCI (National Coordination Communicable Disease Control), Utrecht, NL
4. Academic Medical Center, University of Amsterdam, Dept. of Human Retrovirology, Amsterdam, NL

Abstract

Background- Hepatitis B control in Europe concentrates on antenatal screening to reduce vertical transmission. To reduce horizontal transmission and the pool of infectious individuals, the Municipal Health Service of Amsterdam integrated tracing and immunising of contacts in the antenatal screening program.

Method- An eight year (1992-1999) descriptive study of this public health program, where contacts are tested for serological markers of previous infection, and vaccination is offered to susceptible contacts. Chronically infected contacts are counselled and referred for treatment if justified.

Results- For 738 newly identified women testing positive for the hepatitis B surface antigen, 1219 contacts were reported; 1100 (90.4%) contacts participated, 476 (43%) had serological markers of previous infection, of whom 119 (25%) were infectious. Of 603 eligible contacts, 568 (94%) completed the vaccination series. Country of origin was an independent predictor of contact participation and compliance with completion of the vaccination series. Postvaccination titres for antibodies against the surface antigen were below 10 IU/L in 4.5% of contacts under 30, in 12,2% of those over 30.

Conclusions- Tracing and immunising susceptible contacts of women screened as HBsAg-positive, should be an integral component of any nations HBV control program.

Introduction

Prevention of hepatitis B virus (HBV) infection in babies born to chronically infected mothers is a cornerstone in any nation's strategy to control hepatitis B (1; 2). In the Netherlands, a program of universal antenatal screening for hepatitis B surface antigen (HBsAg) and neonatal hepatitis B immunisation program was tested (3) and introduced in 1989 (4). For the city of Amsterdam, with an estimated 735.000 inhabitants, of whom 37% originate from countries with high HBV endemicity, the Municipal Health Service (MHS) implemented a centralised enhanced program to encompass the approximately 10.000 pregnancies per year(5). In Amsterdam, all HBsAg-positive laboratory results of pregnant women are registered with the MHS, which provides confirmatory testing, hepatitis B immune globulin (HBIg), and vaccination for the neonates. The program is successful in preventing HBV transmission to babies (6). Of women testing positive for HBsAg, 97% originate from countries of high endemicity and come to Amsterdam with partner and/or children with similar background. The HBsAg prevalence for the various groups of women is in accordance with the prevalence found in the country of origin (6). Household members of HBsAg-positive individuals are known to be at increased risk of acquiring HBV infection (7-11), and, phylogenetic tree analysis confirms that it occurs not only by vertical transmission but also through horizontal transmission later in life (12-15). Partners are not only at increased risk for acquiring HBV infection, but, coming from the same background, are also at increased risk of being chronically infected (10). Reduction of the pool of infectious individuals is crucial in managing the population dynamics of hepatitis B endemicity (16), as the often asymptomatic chronically infected family members pose a risk for transmission, within and outside the family, to the children's school mates (11; 17) or later in life to their sexual partners (18). While 151 of 192 WHO-member states (79%) have adopted routine infant or childhood hepatitis B vaccination policies by May 2003 (19), the Netherlands has not, relying on targeted vaccination strategy, with additional efforts to reach individuals at increased risk of acquiring hepatitis B. The Netherlands has no HBV control strategy among immigrants, except for the small groups of asylum seekers, whose children are vaccinated against HBV. Starting in 2003, all neonates of immigrant parents are offered HBV vaccine, but siblings and other household contacts are excluded. Therefore, the MHS Amsterdam has organised enhanced tracing and serologic screening of contacts of all reported HBsAg-positive pregnant women, providing immunisation for susceptible contacts, or, for chronically infected individuals counselling and referral for antiviral treatment if justified. Here we report on this innovative program of centralised enhanced contact tracing, integrated in the antenatal screening program in the years 1992-1999.

Study population and methods

Antenatal screening procedures

As previously described (6), all pregnant women in Amsterdam are tested, with informed consent, for hepatitis B surface antigen (HBsAg), at their first visit to the midwife or obstetrician (scheduled in week 14 of pregnancy). The name and address of HBsAg-positive women are registered with the Municipal Health Service (MHS), which invites them in a letter to come for retesting, counselling and intervention. At the first MHS visit, blood is drawn and sent to the Public Health Laboratory (PHL) for testing to confirm HBsAg (Abbott AxSYM MEIA, Abbott Diagnostics, Wiesbaden, Germany) and to detect antibody to core antigen (antiHBc), e antigen (HBeAg) and antibody to e antigen (antiHBe) (Abbott AxSYM MEIA). Women are regarded as chronically infected if presence of HBsAg has been confirmed by the PHL in the second sample.

At the second MHS visit, information is given about hepatitis B virus infection and the immunisation program. Women are assisted in listing their contacts: (sex)partners, children and other household members. Contacts are then invited to come to the MHS for screening of antiHBc.

In this analysis of the first eight years of the program, we included pregnant women, and their contacts, at first reported pregnancy from 1992 up to and including December 1999 with follow up data until January 1st 2001.

Participation of contacts

Participating contacts are those of whom serological test results are available. We assume that all pregnant women have at least one (sex) partner. Partners living abroad, unknown, anonymous and refusing partners are regarded as non-participants. Participation of children and other household contacts is calculated based on the number of contacts listed by the HBsAg-positive women.

Serological tests in contacts

Participating contacts testing negative for antiHBc are regarded as “susceptible”. Previous hepatitis B vaccination is excluded by interview. In the study period, universal hepatitis B vaccination was not yet implemented in most HBV endemic countries. Contacts testing positive for antiHBc are regarded as “previously infected”. For the latter, the serological hepatitis B status is further evaluated with testing of HBsAg and antiHBs. AntiHBc-positive contacts, who test positive for antiHBs, are regarded as “immune”; those testing positive for HBsAg are regarded as “chronically infected”, infectious individuals or “carriers”.

Preventive action

-- Susceptible contacts are vaccinated (with Engerix™-B, SmithKline Beecham Biologicals, Rixensart, Belgium), scheduled at month 0, 1 and 6. All fully vaccinated contacts are screened for response of antibodies against the hepatitis B surface antigen (antiHBs) at month 7. Individuals with antiHBs titers higher than 10 IU/L are regarded as protected (20). Those with titres below that level (low-responders) receive three revaccinations with retesting of antiHBs.

-- For immune contacts, no further action is undertaken.

-- Chronically infected contacts are counselled and referred to their healthcare provider for further care and antiviral treatment if justified.

Compliance with vaccination program

Susceptible contacts are considered fully compliant if they complete the three dose vaccine series with post-vaccination serology. Those with a low post-vaccination titre (antiHBs <10 IU/L), are fully compliant if they come for three re-vaccinations with again post-vaccination antiHBs evaluation.

Statistical analysis

Routinely, MHS data are entered in a local database. The relevant data for this study were transcribed to a SPSS-9.0 file. Chi-square test was used where appropriate, to compare characteristics between groups. Univariate and multivariate adjusted odds ratios (ORs and AORs), with 95% confidence intervals (95%CI) were calculated using SPSS logistic regression for risk factors in five categories based on participation, susceptibility, chronic infection, compliance in completing the vaccination series, and low postvaccination serological response. We included all available relevant variables in the multivariate analyses. A p-value of less than 0.05 was considered statistically significant.

Results

Participating women

From October 24, 1991 to December 31, 1999 the antenatal screening identified 738 newly registered HBsAg-positive women, 96% of non-Dutch origin. Most women had Turkey as country of origin (171, 24%), followed in descending order by Ghana (17%), Surinam (16%) and Morocco (14%). For most women, the first screening was also for the first pregnancy (384 para 0). Other women came to Amsterdam with one or more children (69 para 1; 90 para 2). Five women were newly registered with the twelfth pregnancy. Of the HBsAg-positive women, 635 had detectable antibodies to e antigen (antiHBe), 86 had e antigen (HBeAg), 5

tested positive for both; in 12 women the HBe status was not evaluated.

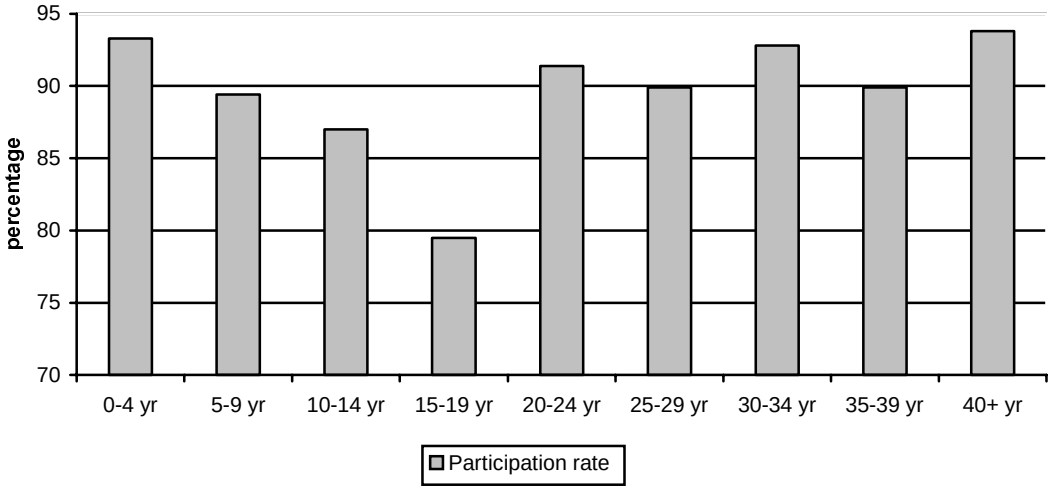
Participating contacts

The 738 women listed 1219 contacts, average 1,7 per index (68 reported no partner nor child; 375 reported 1 contact; and 153 had 2; max 10, median 2). The 738 women reported 644 (sex)partners: 15 partners were living abroad, 31 were unknown, and 48 women refused to give the name of the partner. There were 551 children and 24 other household contacts. Serology was performed for 597 (sex)partners (93%), 483 children (88%) and 22 other household contacts (92%), totalling 1100/1219 participants (90%). Participation was lowest for contacts in the age cohort 14-19 years (chi-square p=0,02).

Partner participation was highest for women from Turkey, also the largest group according country of origin (161/171, 94%). Partner participation was lowest for partners of women from Ghana (62%). Partner participation was lower in recent years (chi-square p=0,02), but not influenced by parity (p>0,6), HBe-status (p>0,82), or age (p>0,13) of the mother, although partners of women of 18 years and younger (n=20) had lower participation (60%, not statistically significant), or age of the contact. In a multiple logistic regression model, including all the above variables, only country of origin of the mother was a statistically significant predictor of partner participation. Compared to the largest group of women, from Turkey, the odds ratio for partner participation was lowest for Ghana (AOR 0.08; 95% CI 0.03 – 0.18), Surinam (AOR 0.14; 95% CI 0.06 – 0.32) and The Netherlands (AOR 0.16; 95% CI 0.05 – 0.55).

Figure 1

Participation of 1100 of 1219 registered household contacts in age cohorts of 5 years of 738 women testing positive for hepatitis B surface antigen (HBsAg).



Serological markers

Among 1100 participating contacts, 476 (43%) had serological markers of previous HBV infection, of whom 119 were chronically infected (carrier rate 11%). Partners more often had markers of previous infection than did children (resp 57% and 28%), and prevalence of previous infection increased with age (Figure 1). The carrier rate was higher among children than partners (resp. 44 and 17 %). Table 1 presents the serological findings of contacts according to the hepatitis B e-antigen status (HBeAg-status) of the mother. The prevalence of previous infection among contacts of women with the e-antigen (HBeAg), is higher in all age groups compared to contacts of women with antibodies to the e-antigen (antiHBe), with exception of children in the age group 15-19 years (Figure 2). Prevalence of previous infection was highest among contacts of women from Ghana (76/123; 62%), while carrier rate was highest among contacts of women from South-East Asia (15/46; 33%). In a multiple logistic regression model, including country of origin and HBeAg status of the mother, age and type of the contact, the infection rate was almost five times higher for contacts of HBeAg-positive women, compared to contacts of antiHBe-positive women (AOR 4,7; 95%CI: 2.9-7.5).

Table 1

Serological markers of hepatitis B infection in 1100 household contacts of 738 women testing positive for the hepatitis B surface antigen (HBsAg), at first pregnancy reported in Amsterdam, categorised by the serological status of the women for the hepatitis B e-antigen: testing positive for the e antigen (HBeAg), or/and antibodies to the e-antigen (antiHBe), or other HBe status.

	N	**Anti-HBc+**		**HBsAg+**		**Carrier rate**
		N	%	N	%	HBsAg/antiHBc
(sex) partners, index HBe+	69	59	85.5	14	20.3	23.7
(sex)partners, index HBe-	514	272	52.9	44	8.6	16.2
Children, mother HBe+	50	24	48.0	15	30.0	62.5
Children, mother HBe-	422	109	25.8	43	10.2	39.4
Others	45	12	26.7	3	6.7	25.0
Total	1100	476	43.3	119	10.8	25.0

Table 2

Numbers and rates of contacts traced, serologically screened, susceptible to HBV infection (negative for antibodies to the hepatitis B core antigen: antiHBc), compliant in three dose vaccination with serological evaluation, and revaccinated if justified, in a sample of 644 partners, 551 children and 24 other household contacts of 738 women testing positive for HBsAg at first pregnancy reported in Amsterdam from 1992-1999.

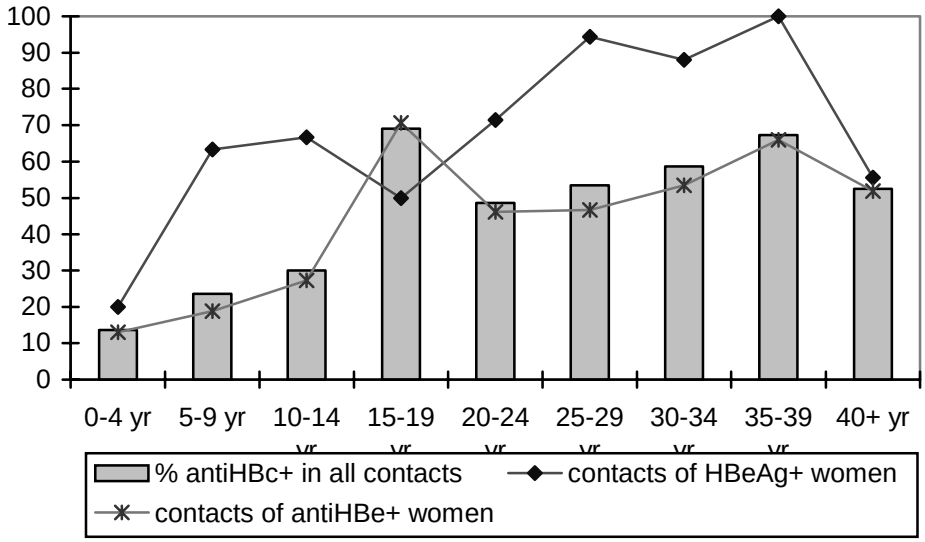
	Registered contacts	Screened		Susceptibles		Completed series	
		N	(%)	N	(%)	N	(%)
Partners	644	595	(84)	258	(43)	223	(86)
Children	551	483	(88)	348	(72)	328	(94)
House-hold	24	22	(92)	18	(82)	17	(94)
Total	**1219**	**1100**	**(90)**	**[1]624**	**(57)**	**[2]568**	**(91)**

[1] Including 21 previously vaccinated contacts.

[2] Of 568 fully vaccinated contacts, 528 (93%) came for serological post-vaccination evaluation (207 partners, 303 children and 16 household contacts); 85% of all susceptible contacts.

Figure 2

Percentage of household contacts in age cohorts of 5 years that were found previously infected in the serological screening (testing positive for antibodies to the hepatitis B core antigen, antiHBc), also related to the e-antigen status of the index women in the household: contacts of women with circulating e-antigen (HBeAg, indicative of high viral load), and contacts of women with antibodies to the e-antigen (antiHBe).



Compliance

Of the 624 contacts testing negative for antiHBc, i.e. “susceptibles”, 21 were already vaccinated. Of the remaining 603 susceptible contacts, 568 (94%) completed the three dose series, 528 (87%) came for post-vaccination titre of antibodies to the hepatitis B surface antigen (antiHBs); 75 without such serological evaluation. Of the 34 contacts that were identified postvaccination with an antiHBs titre below 10 IU/L, 17 (50%) came for a revaccination series with retesting of antiHBs. In total, 92 contacts were not (fully) compliant. In univariate analysis, type of contact (partner/child), age of contact, country of origin, year of registration were all statistically associated with compliance. However, in a multivariate logistic regression, including all above variables, the only predictor of compliance in completing the vaccination series with serological evaluation, was country of origin, with contacts of Surinamese women being less compliant than contacts of Turkish women (AOR 0.3; 95%CI 0.15 – 0.70)

Post vaccination serology

After a three-dose vaccination schedule, 528 participants were tested for antiHBs titres; 34 (6,5%) had a low post-vaccination titre (antiHBs <10 IU/L). Children and young adults under 30, had less frequent low titres (18/397) than adults 30 and over (16/131; resp. 4,5 and 12,2%, p<0.01). In univariate analysis, age of the contact was associated with effect of vaccination, with contacts 15-24 years having more often antiHBS postvaccination titres above 10 IU/L (100%, n=42), than contacts of 40 years and older (84%, n=50). In a multivariate logistic regression, including age of the contact, country of origin, registration year, serological HBe-status of the index women, none of the variables were significant predictors for low serologic response; also not the age of the vaccinated contact. A second serological evaluation was performed after the second series of three vaccines for 17/34 (50%) contacts with an initial low titre, of whom 2 (12%) still showed a serological respons below 10 IU/L (1/6 partners and 1/11 children).

Discussion

This study evaluates an innovative hepatitis B control program in Amsterdam, which integrates the tracing of contacts with an existing program of universal antenatal screening,introduced in 1989 (4; 5). Tracing household contacts of the identified HBV carrier women is mandatory in some, especially Scandinavian, countries, but evaluation data are lacking, or show unsatisfactory results (21). We find high participation (90%) and high vaccination rates in susceptible contacts (91%) in these multi-cultural contacts at high risk for acquiring hepatitis B. Our approach can be applied elsewhere.

High-risk groups

In the Netherlands and other areas of low endemicity, universal childhood hepatitis B vaccination appears not to be cost effective (22). In addition to universal antenatal screening hepatitis B control relies in the Netherlands on vaccination programs targeted at high-risk groups (23) and source and contact tracing around reported cases of acute hepatitis B. Antenatal screening in Amsterdam finds the highest carrier rates among inhabitants of foreign origin (6). Families settled in our country coming from the former Dutch colonies (Surinam and, less often, Indonesia), from sub-Saharan Africa largely as asylum seekers, or from northern Africa, following recruitment as cheap labour force in the sixties and seventies of the twentieth century. Their homelands have high endemicity for hepatitis B where implementation of antenatal screening and neonatal vaccination is often in its infancy.

We find a relatively low number of contact children, because most families are young. Our data show high HBV infection rates (62% for contacts of women from Ghana) and high carrier rates (33% for contacts of women from South-East Asia). As expected, the prevalence of markers of infection (antiHBc) increases with age, indicative of ongoing intrafamilial horizontal transmission, not only to children (11; 24; 25), but also to spouses (26). We have no explanation for the peak prevalence in children aged 15-19 years, especially for contacts of antiHBe-positive women. Because this specific cohort had the lowest participation rate (79,5%) and comprises only 58 youngsters, we consider this as an aberration due to small numbers.

It is the challenge of every public health practitioner to increase participation and compliance to preventive programs among the groups at highest risk (27), who, very often, are the most difficult to reach (28). The first eight years of the Amsterdam program served an easily accessible high-risk population with good participation (90%). Even with high infection rates, we found that 57% of contacts were still HBV susceptible, and these had high compliance for immediate protection by vaccination, as 91% completed the three-dose series. Among the 1100 contacts, we also found 11% silent chronically infected individuals, who were motivated to receive hygienic advice and treatment. Treatment of such individuals is not only in their personal interest, but reduces the pool of infective individuals, which is crucial to managing the population dynamics of hepatitis B endemicity (29). .

Starting March 2003 hepatitis B vaccination is offered in the Netherlands to newborn infants of parents originating from countries of intermediate and high endemicity. There is, however, no catch-up program for older siblings. Screening partners and children of HBsAg positive pregnant women remains an essential component of the hepatitis B control program.

Integrated hepatitis B control

With the first descriptive epidemiological studies in the 1970s, it became known that hepatitis B clusters within families. Our serological data of household contacts of chronically infected women, confirming a proportion of 43% previous infections, are in the range of these early studies (7; 30-32) where infection rates range from 33 to 73%. Since the introduction of a safe and effective vaccine in the 1980s (33; 34), the public health consequence should have been to screen these contacts and target susceptible contacts for vaccination (10). Such screening was specifically recommended in the USA as early as 1985 (35). A recently published first evaluation, shows that its most compliant study area, was Dallas, with 61% of contacts screened, and 59% of susceptibles vaccinated (36). According to the authors, Dallas differs favourably from the three other USA study sites, because of its specific centralised self-contained program, which includes

home visits. Our program integrates, as in that program, contact tracing with antenatal screening. Home visits are used by us for neonatal immunisation, but might raise awareness and motivation in contacts.
Centralised hospital based neonatal immunisation programs show high uptake (37), but such programs are not equipped to trace contacts. If integrated into comprehensive HBV control these programs offer an ideal opportunity to reach more high risk individuals, who present themselves at the doorstep of our institute, and, as family members of a new born, are motivated to participate.
We agree with public health practitioners in countries of low HBV endemicity, that antenatal screening and targeting might be sufficient to control HBV (38). But, with added tracing of contacts it can protect older children and adults at high risk for infection, and reduce the pool of infective individuals.

Serological evaluation after vaccination
Others have found that post-vaccination titres decline with increasing age at vaccination, but not until about the age of 30 years (39-42). Also our data showed 12,2% low-responders, i.e. with antiHBs titre below 10 IU/L, for 30 years and older, and 4,5% for younger than 30. In situations of continuous exposure, routine serological post-vaccination evaluation should be continued, with revaccinations for low-responders that results in sufficient titres for most (88%).

In the animal world, hepatitis B co-speciated with various primates and rodents in the America's. Hepatitis B has a high prevalence amongst aboriginal, Amazonian Indians, and Inuit populations. Maybe hepatitis B is, with syphilis, another example of export of a disease from the New to the Old World (as opposed to smallpox, measles, and influenza, that decimated populations in the New World due to importation from the Old World)
Holmes EC, Zanotto PM. The ecology and evolution of human hepatitis viruses. [Edited]

Conclusion

We were encouraged to see that 90% of contacts at high risk for acquiring hepatitis B, came to the MHS and were protected or counselled as required. As 91% of the susceptible contacts completed the three dose vaccination series, we conclude that contact tracing, integrated with antenatal screening, improves motivation and should be an inextricable component of any nation's HBV control program, regardless existing universal childhood vaccination..

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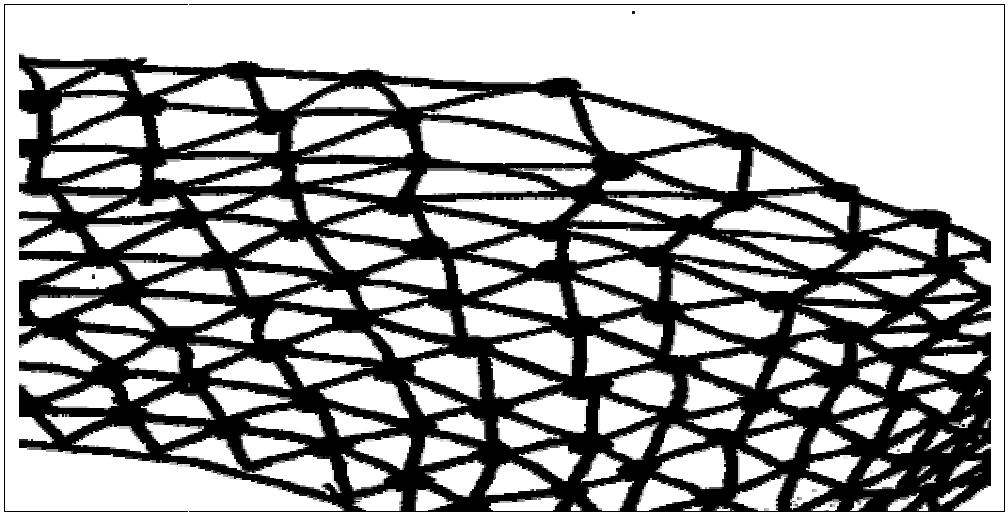
Chapter 4

Results of an enhanced-outreach program of hepatitis B vaccination in the Netherlands (1998-2000) among men who have sex with men, hard drug users, sex workers and heterosexual persons with multiple partners

Jim E. van Steenbergen (1,2) and the Working Group Vaccination High-risk Groups Hepatitis B for The Netherlands*

* See Appendix



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1. GG&GD (Municipal Health Service), Dept. of Infectious Diseases, Amsterdam, the Netherlands (NL)
2. LCI (National Co-ordination Communicable Disease Control), Utrecht, NL

Abstract

Background- Besides universal antenatal screening, the Netherlands relies on targeted vaccination for hepatitis B control. In 1998 the Ministry of Health funded a two year pilot project directed at vaccinating behavioral high-risk groups for sexual and drug related hepatitis B transmission.

Methods- In seven participating Municipal Health Service-areas (MHS) hepatitis B vaccination (HBVax MSD) was offered without costs to men who have sex with men, drug users and heterosexuals with multiple partners, including commercial sex-workers. Participants were included during the first 18 months of the project (October 1998 – May 2000). Second and third vaccinations were given up to October 2000. Four regions, designated as intervention areas, started enhanced recruitment. Depending on the local situation this was done either through intermediary caretakers or by the MHS staff: methadone and STD-clinics included the vaccinations in the standard operating procedure; the MHS deployed opinion and peer leaders to recruit participants or peers by using a snowball method; outreaching vaccination was carried out in non-medical low threshold places. Intermediaries and drug users were offered an incentive. Three areas, designated as control, used flyers only to invite people to come for free vaccination at the regular opening hours of the MHS.

Results- In 18 months 13.808 people from high-risk groups entered the program. This is a coverage of 63% of the targeted population in the intervention areas and 23% in the control areas. The enrolment of drug users remained far behind expectation (19% in intervention regions, 4% in control regions). The enrolment of the heterosexual population (64%) was satisfactory. This was achieved partly due to structural uptake of vaccination in the standard operating procedure in the Amsterdam STD-clinic, partly by multiple outreach efforts. There were regional differences in success and failures of various recruitment strategies.

Conclusions- If given sufficient facilities, the MHS in the Netherlands are able to reach and enrol a limited number of high-risk individuals for hepatitis B vaccination; more time is needed to implement the program, especially for hard drug users. Successful strategies in one region are not uniformly transferable to other regions. Although feasible, this high-risk approach is not able to reach sufficient numbers of high-risk individuals to protect the population against hepatitis B. However, even with universal vaccination these high-risk groups have to be attended to for the coming decennia. The experience gained with the various strategies will be deployed by all other MHS in The Netherlands, and successful strategies can be applied elsewhere in the industrialised world.

Introduction

The Netherlands is a country of low endemicity for hepatitis B. The reported incidence of acute hepatitis B varied in the last decade from 1.4 to 1.8/100.000 inhabitants [1], although a GP sentinel system suggests that actual incidence is 3.2 – 6.1/100.000 [2]. To control transmission, patients with acute hepatitis B are referred to their Municipal Health Service (MHS) facility, where interviews ascertain the probable mode of transmission and preventive action is undertaken. In a random sample of 140 cases in 1999-2000, the probable mode was homosexual contact 30%, heterosexual contact 29%, drug-use 21%, and other or unknown 20% [1].
In 1996, the Dutch Health Council advised the Ministry of Health, Welfare and Sports to vaccinate three specific groups at high risk for hepatitis B: men who have sex with men (MSM), drug users (DUs) and heterosexuals with multiple partners [3]. In 1997, the Ministry offered financial incentives and free vaccine to any MHS facility submitting a plan to vaccinate these groups during a pilot period of eighteen months. The goal was to evaluate strategies to enhance recruitment for hepatitis B vaccination and improve compliance. Seven MHS areas responded, of which we studied four as intervention regions and three as control regions. Here we report on the strategies, the population that started and completed the vaccination series, and the cost in staff and money. Effective strategies will be used in a nation wide implementation of the program and might be used in other countries of low endemicity.

Participants and methods

High-risk populations
High-risk groups for hepatitis B were defined as men who have sex with men (MSM), users of hard drugs but not party drugs like ecstasy (DUs), and heterosexuals with multiple partners. This last group included male and female sex workers (SWs) and persons treated at sexually transmitted disease (STD) clinics. The estimated MSM population in the seven regions was based on Sexual Health Surveys [4,5], with DUs based on DU registries and SWs based on two inventories and various surveys [6,7] plus data from the STD foundation. The number of clients of STD clinics were based on the annual reports of these clinics. Estimates were reviewed by experts and by advocacy and support organisations and the highest estimate chosen in case of inconsistencies.

Target high-risk population

The participating seven MHS facilities estimated their target population for the 18-month enrollment period based on their recruiting methods in the pertinent groups. When there was data on the prevalence of HBV markers in the groups, the target population was based on an estimate of the susceptible population.

Screening and vaccination

Starting October 1998, hepatitis B vaccination (HBVax MSD) was offered free in the seven MHS areas on the normal schedule of month 0, 1 and 6. Enrollment ended in April 2000, but second and third doses were offered until October 2000. On receiving the first dose, participants were tested for total antibody to hepatitis B core antigen (anti-HBc). A chi-square test was used to compare the susceptibility of high-risk groups, defined as negative anti-HBc rates, in the intervention versus control areas; susceptibility was defined by the group rate of negative anti-HBc findings. Participants learned test results on returning for HBV-II. Those positive for anti-HBc were referred to their healthcare provider for complete serological hepatitis B screening and counselling, with treatment on findings of chronic infection. Contacts of chronically infected patients are routinely traced by the MHS and vaccinated if susceptible. We referred participants that opted for post-vaccination screening for antibodies to the hepatitis B surface antigen (anti-HBs) to their healthcare provider.

Recruitment

Flyers promoting hepatitis B vaccination were developed and distributed in all seven areas. They described local vaccination resources in Dutch, Spanish, English, and German.

Enhanced outreach was undertaken in the four intervention areas (Table 1a), of which one was metropolitan (Amsterdam, population 750.000), two urbanised (295.000 and 185.000, respectively) and one rural (198.000). When possible, vaccination was integrated into medical and non-medical services already offered to high-risk groups. Its availability was publicized using existing communication lines among support and advocacy groups. General media was not used, since free vaccine was provided in only seven of the country's 45 MHS areas, serving 16.5% of the total population (intervention 9%, control 7.5%).

In the metropolitan intervention area, relevant healthcare providers were invited to promote and administer the vaccine, and GPs received 5,- Euro for each vaccine administered. In STD and methadone clinics, screening and vaccination was integrated into the usual routine, with vaccine offered to all those who knew the Dutch or English language, had a postal address, and planned at least six months' residence in Amsterdam. STD clinic staff was expanded as part of the

pilot. Physicians working at correctional facilities were involved and assisted by MHS nurses in blood-taking and initial registration. Advocacy and support groups were asked to participate by directing high-risk individuals to vaccination sites. Sex workers were approached by MHS nurses through an existing program of periodic visits to brothels.
In the urbanised intervention areas, outreach strategies were tailored to each high-risk group, with vaccination performed in various locations. Opinion leaders among MSM were invited to advocate the program in meeting places such as gay bars, and MHS nurses provided vaccination on the spot. For DUs, vaccination was performed at sites for methadone outlet, needle exchange, and homeless shelter. For SWs, it was offered at brothels and zones of street prostitution.
In the rural intervention region, DUs were recruited through the methadone program and the "snowball method," by which DUs are informally trained, then offered 5,- Euro for each fellow DU they recruit. Information and first vaccination were given to MSM at sexual meeting places and to SWs at brothels.
The enhanced outreach above was compared with more passive outreach in three MHS control areas, where free vaccine was publicized only by distributing flyers to healthcare providers. It was administered only at MHS during regular office hours. Control areas included an urbanised area (population 288.000) and two mixed urbanised-and-rural areas (560.000 and 373.000, respectively).

Table 1a

Size of general, high-risk, and targeted populations and number of participants and percentage participants of target population per Municipal Health Service (MHS) in the four intervention and three control areas in the 18-month enrollment period of the pilot hepatitis B vaccination program, The Netherlands.

Intervention areas	General	High-risk	Target	Partici-pants	% target	% high risk
Metropolitan	750.000	52.000	19.050	11.935	63	23
Urbanised	295.000	4.300	850	530	62	12
Urbanised	185.000	2.600	640	496	78	19
Rural	198.000	2.400	300	223	74	10
Sub-total intervention	**1.428.000**	**61.300**	**20.840**	**13.184**	**63**	**22**
Control areas						
Urbanised	288.000	3.730	260	153	59	4
Mixed urbanised and rural	373.000	3.000	750	150	20	5
Mixed urbanised and rural	560.000	3.270	1.760	321	18	10
Sub-total control areas	**1.221.000**	**10.000**	**2.770**	**624**	**23**	**6**

Table 1b

Size of high-risk, and targeted populations and number of participants per high-risk group in the four MHS intervention areas and three MHS control areas, during the 18-month enrollment period of the pilot hepatitis B vaccination program, The Netherlands.

In intervention areas	**Population**	**Target**	**Participants**	**% target**	**% high risk**
Men having sex with men	28.080	5.500	3.523	64	13
Drug users	8.120	5.600	1.068	19	13
Sex workers	8.600	1.440	652	45	8
Heterosexual STD clinic visitors	13.500	8.300	7.941	96	48
Sub-total intervention	**58.300**	**20.840**	**13.184**	**63**	**22**
In control areas					
Men having sex with men	7.240	950	426	45	6
Drug users	2.000	1.350	57	4	3
Sex workers	460	340	79	23	17
Heterosexual STD clinic visitors	300	130	62	48	21
Sub-total control	**10.000**	**2.770**	**624**	**23**	**6**

Compliance

We define a compliant participant as a starter who completed the three-vaccination series. The STD clinic in Amsterdam sent one reminder to anyone not coming for HBV-II or -III within one month of its due date. However, if they did not arrive within another month, it closed their record. In the urbanised intervention areas, MSM and STD clinic visitors received a written or telephonic reminder, depending on preference. They were given a key holder with vaccination card as an incentive, while DUs could opt for money (5,- Euro). In the rural area, DUs scheduled for HBV-II or -III were reminded through the methadone program. Individuals vaccinated at other sites were reminded by staff at subsequent visits. Compliance, or uptake of HBV-III, was compared between the combined intervention areas and the combined control areas with a chi-square test. We analysed compliance among the high-risk groups and compliance per vaccination facility in two similar logistic regression models.

Funding and costs

The Dutch Ministry of Health, Welfare and Sports funded the project and enabled the following activities: development and distribution of flyers, purchase and distribution of vaccines for the intervention areas, serological tests, co-ordination of the project, appointment of additional staff in STD clinic and MHS facilities, development and distribution of a data registration system. The project received a financial grant from Aventis Pasteur MSD, The Netherlands, that enabled delivering hepatitis B vaccine free of charge in the control areas.

Project cost was calculated per completed vaccination series and also per protected (fully vaccinated, infected, or immune) individual.

Results

High-risk population

The high-risk groups in the four intervention areas were estimated to total 61,300 persons, with a target population for the 18-month enrolment period of 20,840 (Table 1a), or 34% of the high-risk population. The high-risk population in control areas was estimated to be 10,000 individuals, with 2,770 (28%) targeted. A total of 13,808 high-risk persons entered the program, 13,184 in the intervention regions (63% of those targeted, and 22% of the broader high-risk population) and 624 in control regions (23% of those targeted, and 6% of the broader population). In the intervention regions, 64% of targeted MSM but only 19% of targeted DUs received the first vaccination dose (Table 1b). The SW programs included 45% of the targeted number, thus reaching only 8% of the broader SW population. In control areas, 23% of targeted SW population (17% of the broader population) received the first dose.

Recruitment strategies

In the intervention areas, members of all three high-risk groups were processed through STD clinics, correctional facilities, GP practices, and existing outreach activities. Of all participants in these areas, 69% entered the program through STD clinics, including 1466 MSM, or 42% of MSM participants in these areas. Correctional facilities recruited 2% of all participants, contributing 3% of DUs, and 10% of SWs. Of the 435 GPs in Amsterdam, 70 agreed to participate, but only 11 vaccinated 359 individuals, of whom most were MSM, or 9% of MSM participants. Recruitment through existing outreach for MSM contributed 18% of MSM, and MHS recruitment bringing 26%.

Of DUs, methadone clinics enrolled 44%, while 3% came through correctional facilities, and only 1% through STD clinic or GP. In Amsterdam, methadone clin-

ics are integrated with MHS, where most DUs were vaccinated, but only 14% of the city's targeted DU population (686/5.000) entered our program. In the two urbanised and one rural intervention regions, methadone clinics recruited 51, 70 and 36% of the targeted populations, respectively. Of SWs, 33% were vaccinated at brothels by MHS nurses, 33% at the local MHS, and 15% of sex workers at the STD clinic.
Strategies involving non-medical sites typically started vaccination on the spot and continued the series at the local MHS. Successful strategies included, for MSM, the personal approach by MHS nurses at meeting places and through bartenders serving as opinion leaders; for DUs, working through the field workers serving them. In urbanised regions, newspaper notices inserted among personal advertisements brought MSM for vaccination but failed to reach them in the metropolitan region. The snowball strategy for DUs failed, bringing not one to the program in any region.

Screening
In the intervention areas, screening for anti-HBc on the first vaccination found 14% of participants to be previously infected, compared to 3% in control areas (Table 2a, chi-square p<0,001). In both areas, DUs were more often found positive than other participants (Table 2a, chi-square p<0,001). Screening identified 114/1910 (6%) high-risk individuals previously unknown to be chronically infected, including 19 MSM, 13 DUs, 11 SWs (including 2 male) and 71 STD clinic visitors.

Table 2a

Number screened, number susceptible (%), and number of participants that completed second (HBVac-II) and third (HBVav-III) hepatitis B vaccination dose per high-risk group in the combined intervention areas and combined control areas in the 24-months duration of the pilot hepatitis B vaccination program in The Netherlands.

Intervention areas	**Number screened**	**Anti-HBc negative[a]**	**(%)**	**HB-Vac-II**	**%**	**HB-Vac-III**	**%**	**Odds ratio[d]**	**95% CI**	
MSM	3.523	2.899	82	2.569	89	2145	74	1,63	1.51	1.77
Drug users	1.068	740	[b]69	615	83	430	58	0,71	0.62	0.81
Sex workers	652	528	81	379	72	231	44	0,58	0.49	0.68
Heterosexual STD-clinic visitors	7.941	7.107	89	5.186	73	3.875	55	1.00	-	-
Sub-total intervention areas	**13.184**	**11.274**	**[b]86**	**8.728**	**77**	**6.681**	**[c]59**			
Control areas										
Men having sex with men	426	419	98	396	95	331	79	3,06	1.77	5.30
Drug users	57	49	[b]86	40	82	24	49	0,64	0.31	1.32
Sex workers	79	79	100	56	71	35	44	0,70	0.36	1.36
Heterosexual STD-clinic visitors	62	56	90	52	93	33	59	1.00	-	-
Sub-total control areas	**624**	**603**	**[b]97**	**544**	**90**	**423**	**[c]70**			

a. negative anti-HBc is regarded as no previous infection with the hepatitis B virus

b. percentage susceptibles in intervention area lower as compared to control areas (χ^2, $p<0.001$)

c. compliance in intervention areas did not differ from control areas (χ^2, $p>0.05$)

d. Odds ratio with 95% CI of the compliance with the HBVac-III of the high-risk group in reference to category heterosexual visitors STD-clinic

Table 2b

Number screened, number susceptible (%), and number of participants that completed the second (HBVac-II) and third (HBVac-III) hepatitis B vaccination dose per facility in the combined intervention and combined control areas during the 24-months duration of the pilot hepatitis B vaccination program in The Netherlands.

Intervention areas	**Number screened**	**Anti-HBc-[a]**	**(%)**	**Number HBVac-II**	**(%)**	**Number HBVac-III**	**%**	**Odds Ratio[d]**	**95% CI**	
STD clinics	9072	7868	87	5784	74	4466	56	1.00	**-**	**-**
Correctional facilities	263	231	88	181	78	83	37	**0.48**	0.37	0.62
General practitioners	359	323	90	284	88	229	71	**1,82**	1.46	2.26
Gay-clinics	638	541	85	488	90	377	70	**1,49**	1.27	1.75
Methadone clinics	494	359	73	303	84	214	59	**0.79**	0.66	0.95
Sex clubs	406	369	91	271	73	177	48	**0.80**	0.65	0.97
MHS	1859	1515	81	1378	91	1088	72	**1.46**	1.32	1.61
Other	93	68	73	60	88	47	69	1,05	0.70	1.59
Sub-total intervention areas	**13.184**	**11.274**	**86**	**8749**	**78**	**6681**	59			
Control areas										
MHS	546	537	98	486	91	390	73	**3.40**	2.10	5.54
Other	78	66	85	58	88	33	50	1.00	-	-
Sub-total control areas	**624**	**603**	**97**	**544**	**90**	**423**	70			

a. negative anti-HBc is interpreted as no previous infection with the hepatitis B virus

d. Odds ratio with 95% CI of the compliance with the HBVac-III at facility compared to reference, i.e., STD clinic for intervention and other facilities for control areas.

Compliance

The combined high-risk groups in the intervention regions did not differ significantly in compliance from combined groups in control areas (Table 2a, chi-square p>0.05). In the former, compliance was higher among MSM than among STD clinic visitors (OR 1,63; 95%CI 1,51- 1,77). Among DUs and SWs, compliance was lower (respectively, OR 0,71; 95% CI 0,62-0,81 and OR 0,58; 95%CI 0,49-0,68). In control areas too, MSM had better compliance than STD clinic visitors.

As shown in Table 2b, GPs, MHS facilities, and gay clinics reported more patient compliance (in declining order) than did STD clinics, brothels, methadone clinics, and correctional facilities (in declining order).

Costs

Excluding cost of vaccines, the cost of their administration was 590.000,- Euro. Per completed vaccination series, cost was 83.18 Euro; per protected individual, 65.45 Euro. Staff hours required per completed series varied in the intervention areas from 1.32 hours in the metropolitan area to 7.01 hours in one urbanised area. In the rural area, 3.05 hours were needed per completed series.

Discussion

To protect high-risk individuals against hepatitis B, increased vaccination is needed using various approaches [8,9]. In The Netherlands, MHS facilities operate locally and can reach high-risk groups in their area. In our pilot program involving 7 areas, 4 intervention areas with enhanced recruitment had better results (63% of targeted population, 22% of total high-risk population) than 3 control areas (23% of targeted population, 6% of high-risk population). Only 3% of participants in control areas were anti-HBc-positive, versus 14% in the intervention areas (Table 2). This finding reflects the selection of individuals with less risk in the control areas (the “worried well”) and may explain the higher compliance there. Given these factors, it is encouraging that compliance among the high-risk groups did not differ significantly from intervention to control areas.

Of all hepatitis B patients reported in recent years, more than half had been through STD clinics or correctional facilities before the infection was diagnosed [10], but these venues missed opportunities for preventive vaccination [11,12]. We and others [13,14] have shown that with additional incentives, STD clinics can integrate vaccination into their routine. Our finding of low compliance at the Amsterdam STD clinic was due to a coincidental two- to four-fold increase in STD diagnoses [15,16] that caused overburdened staff to close records on per-

sons not responding to HBV-II reminders within one month.

In Dutch prisons, high-risk behaviour is low [29], but vaccination there would help prevent HBV transmission after detention [10]. As in comparable countries [28], about 12% of prisoners in The Netherlands were anti-HBV-positive. Compliance with our vaccination program was low, largely because few were detained long enough to complete the series.

In the Netherlands and other industrialised countries [17], the level of hepatitis B vaccination in MSM remains low [18] despite the efficacy of various strategies [19,20]. We recruited MSM successfully through bartenders of gay bars, an approach similar to that of the Gay Men's Task Force in Glasgow, where higher levels of hepatitis B vaccination were found among MSM reached by peer educators [21]. Most of our participating GPs found it hard to identify high-risk individuals among their patients and, having identified them, were hesitant to discuss hepatitis B vaccination with patients visiting for unrelated reasons. However, a group of eleven gay-friendly GPs in Amsterdam enrolled 9% of participating MSM, and once these GPs managed to broach the subject, their clients were most likely to complete series, due to their professional attention.

In general, vaccine delivery to DUs is poor [22], but programs to vaccinate high-risk individuals in clinical or non-clinical settings have effectively reached DUs [14]. Our recruitment of this group was delayed by the need to organise venipuncture staff at methadone clinics, and most DU participants entered our program during its last six months. Our yield was therefore not high, but our strategy was ultimately successful, and DU compliance matched that of other integrated projects [23, 24] and exceeded that of research settings [25].

The integration of SW vaccination into routine visits to brothels by MHS nurses worked well for us and for researchers in Belgium and Austria [13, 26, 27]. However, since the SW population is migratory, completing the vaccination series was often impossible.

The average cost of vaccine administration (excluding vaccine) was higher if calculated per completed series (83.18 Euro) than per person protected (65.45 Euro), since some non-vaccinated individuals are protected by previous infection. Also, protection can be assumed for some persons who receive only one or two doses. Besides, through self-selection and selection by health care professionals, few previously infected individuals participated, as shown by the relatively low percentage of HBV markers we found. For example, the prevalence of HBV markers in DUs of the Netherlands reportedly varies from 68% to 81% [30,31] but was 31% among our DU participants.

Reduction of the pool of infective individuals is crucial to managing the population dynamics of hepatitis B endemicity [34]. Our project thus had public health benefits, since 114 individuals with previously unknown chronic infection were de-

tected at the first visit. They were referred for MHS evaluation and possible treatment; their intimate contacts were screened and offered vaccination if seronegative.
The overall vaccine coverage after 18 months of enhanced outreach was nevertheless disappointing. Only 22% of the high-risk population was recruited. Of susceptible participants, 59% completed the vaccination series, raising the existing protection rate for the high-risk population obtained through previous infection, with only 14%. A vaccine giving full protection after two or even one dose would have raised this rate to 17 or 22%. Higher levels of enrolment and full vaccination could be expected if our project were expanded and publicised extensively, with nation-wide implementation [32]. In fact, the Dutch Ministry of Health, Welfare and Sports plans such implementation, to begin November 2002, but coverage is still unlikely to reach the 63% required to stop endemic circulation [33]. Although The Netherlands provides good access to health care for various high-risk groups, MHS facilities will not reach all individuals at high risk for acquiring hepatitis B. Universal vaccination would be needed to eliminate the disease, even in countries of low endemicity like The Netherlands. This projection argues that acceptance would be similarly poor for any vaccine developed against HIV. Even with universal vaccination, groups at high risk for HBV infection will need special attention over the coming two decades, i.e., until vaccinated birth cohorts have reached the high-risk age. We found no strategy to be successful in all regions but have produced and tested an array from which MHS areas can choose and adapt to their existing services and their particular high-risk population. Successful strategies might be beneficial in other countries of low endemicity.

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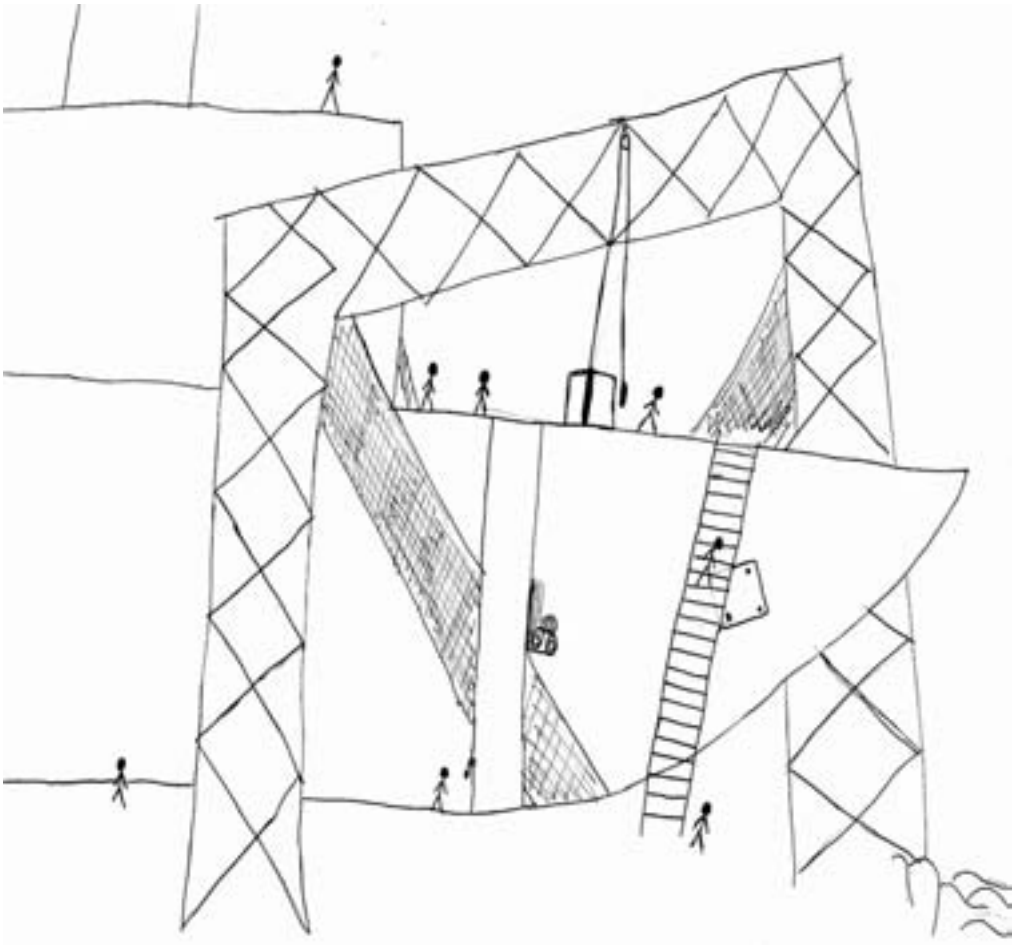

The description by Lürman in January 1885 is a precise and careful account of an outbreak of hepatitis B related to smallpox revaccination. Lürman elegantly relates the jaundice to the use of human lymphe in smallpox inoculations of workers in a shipyard:

Die von October 1883 bis April 1884 hier in Bremen beobachtete Icterusepidemie betraf das Personal der Actien-Gesellschaft „Weser“. Dies Etablissement (Schiffsbau, Maschinenfabrik und Eisengerei) ist am westlichten Ende der Stadt auf dem rechten erhöhten Weserufer gelegen.
Eine Icterusepidemie. Mitgetheilt von Dr. Lürman in Bremen. Berliner Klinische Wochenschrift. No 2, 12 Januar 1885, pp 20-23.

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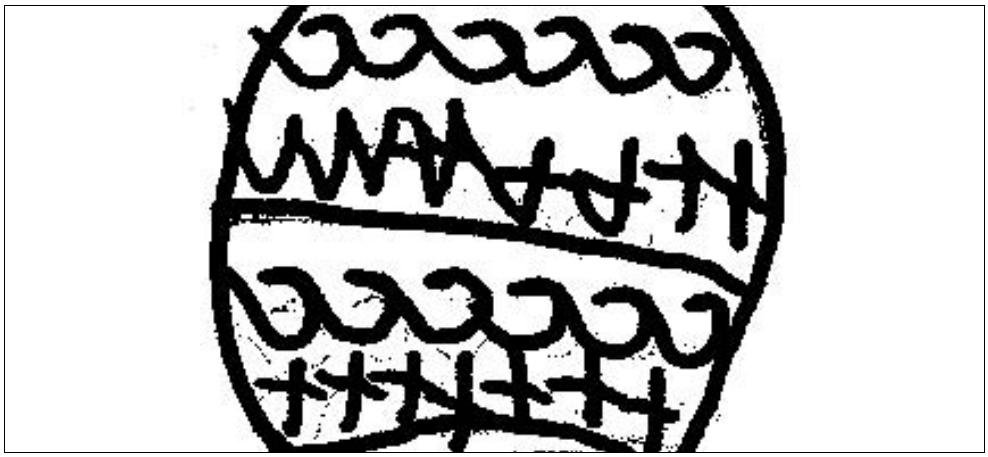
Appendix

The Working Group Vaccination High-risk Groups Hepatitis B for The Netherlands
Academic Medical Centre, University of Amsterdam, Dept of Human Retrovirology: Roel Coutinho. GG&GD Amsterdam: Han Fennema, Anneke van den Hoek, Ruud Lensen, Dieke Mulder-Folkerts, Merel Schutten, Evelien Siedenburg, John de Wit. GGD Hart voor Brabant: Els Jacobs, Harry van Krugten. GGD Oostelijk Zuid Limburg: Christian Hoebe. GGD Rivierenland: Helma Ruijs. GGD Zuidelijk Zuid Limburg: Maria Jansen. Hulpverleningsdienst Groningen: Karin Droog. LCI: Marieke van de Burgt, Quita Waldhober

Chapter 5

Molecular epidemiology of hepatitis B virus in Amsterdam 1992-97

Jim E. van Steenbergen (1,2), Bert H.G.M. Niesters(3), Eline L.M. Op de Coul (1,5), Gerard J.J.van Doornum (4), Ab D.M. Osterhaus (3), Anne Leentvaar-Kuijpers (1), Roel A. Coutinho (1,6), Anneke J.A.R. van den Hoek (1)



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1. GG&GD (Municipal Health Service), Dept. of Infectious Diseases, Amsterdam, The Netherlands (NL)
2. LCI (National Co-ordination Communicable Disease Control), Utrecht, NL
3. Erasmus Medical Centre, Department of Virology, Rotterdam, NL
4. GG&GD, Public Health Laboratory (Streeklaboratorium voor de Volksgezondheid) Amsterdam, NL, now at (3)
5. Now at the RIVM (National Institute of Public Health and the Environment), Bilthoven, NL
6. Academic Medical Centre, University of Amsterdam, Dept. of Human Retrovirology, Amsterdam, NL

Abstract

Aims- To gain insight into the spread of hepatitis B among various risk groups in Amsterdam.

Methods- A six-year (1992-1997) retrospective DNA sequencing study was performed of isolates from stored sera from reported primary cases of acute hepatitis B infection. Cases were classified according to risk behavior, as determined in interviews. Of available serum, a selected region of hepatitis B-virus-DNA was amplified and sequenced. The nucleotide alignments were subjected to phylogenetic tree analysis.

Results- When nucleotide alignments were subjected to phylogenetic analysis, the strains of 54 isolates, 26% of the 204 reported primary cases, clustered in five genotypes: A, C, D, E and F. In genotype A, we identified a cluster related to men having sex with men. In genotype D, two subclusters could be identified, one, related to injecting drug use and another, related to the Moroccan population in Amsterdam. The remaining strains showed a high genetic variability within three different genotypes: F, E and C. Of the 14 identical isolates in the "homosexual men cluster", one was isolated from a female heterosexual. Of the 14 identical strains in the "drug users strain" six were from non-drug using heterosexual active individuals. In the cluster of twelve isolates related to hepatitis B-endemic areas probable modes of transmission were varied.

Conclusions- Sequence analysis provides important insight into the spread of hepatitis B among various high-risk groups. The analysis indicates that the prevention strategy in The Netherlands, fails to stop transmission of hepatitis B from chronic infected individuals originating from hepatitis B-endemic countries.

Introduction

In the Netherlands, approximately 250 patients with acute hepatitis B are reported annually. Between 1993 and 1998, the reported incidence varied from 1.4 to 1.8/100.000 inhabitants [1], but a general practitioners sentinel system suggests that actual incidence is two to six times higher (incidence ranging from 3.2 – 6.1/100.000 [2]. Patients are reported with the probable mode of transmission as ascertained by the reporting physician. In 1998 the probable mode of transmission was heterosexual contact in 32%, homosexual contact in 19% and injecting drug use in 3%. Since social and psychological barriers hamper open discussion of present and former risk behavior, the probable mode of transmission was often unclear and registered as unknown. The percentage registered as unknown declined from 61% in 1993 to 46% in 1998 [2].
In Amsterdam, the incidence of reported acute hepatitis patients varied in the same period from 3.2-6.1/100.000, and percentages for the probable modes of transmission were 36% men having sex with men, 27% heterosexual contact, 11% injecting drug users, 6% other transmission and 20% unknown. This difference from the nation wide percentages reflects the risk populations in Amsterdam, and another important factor is the long-standing tradition of the Municipal Health Service in source- and contact-tracing. The effort and experience of the Municipal Health Service with history-taking in tracing risk behavior results in a lower percentage of unknown cases. Nevertheless, even in Amsterdam misclassification is possible, and the percentage unknown is still high. To gain more insight into the spread of hepatitis B among various risk groups and to detect possible new risk groups, we conducted a pilot study. Its aim was to find out if isolation and sequence analysis of virus strains can assist in ascertaining the source of community acquired acute hepatitis B infection. This retrospective sequence analysis was performed on HBsAg-positive sera, stored in the Public Health Laboratory, with regard to patients diagnosed with acute hepatitis B and reported to the Municipal Health Service from 1992 to 1997. The molecular epidemiology of the acute disease in Amsterdam, identifies three clusters related to specific populations and behaviors with high risk for hepatitis B.

Participants and methods

Participants
From 1992 to 1997, 204 index patients with community acquired acute hepatitis B were reported as primary cases to the Municipal Health Service in Amsterdam. Reporting criteria are clinical signs and symptoms in combination with laboratory

confirmation of acute infection as measured by the appearance of hepatitis B surface antigen (HBsAg) or the presence of type M immunoglobulin antibodies to the hepatitis B core antigen (antiHBc-IgM). The Municipal Health Service approaches all reported patients for active surveillance including source- and contact-tracing, partner notification, preventive intervention consisting of immunization of susceptible contacts at risk, and hygienic advice. Information on risk behavior during the three to six months preceding each infection is obtained by an experienced public health nurse in one or more, in-depth interviews. Individuals are classified with an algorithm by probable mode of transmission. The first group includes people with specific hepatitis B-risk behavior: men having sex with men, individuals with unprotected heterosexual contact with new or multiple partners or injecting drug users. Transmission to persons without such behaviors is classified as household transmission, health care-related, or unknown. The transmission to an individual without specific hepatitis B-risk behavior but with a household contact who is identified as carrier of hepatitis B surface antigen (HBsAg), is classified as household transmission. Without hepatitis B-risk behavior and without HBsAg positive household contacts, the transmission is classified as health care related, if invasive procedures were performed in the six months preceding infection. If none of these risks are identified, the transmission is classified as “unknown”. Besides risk behavior, other data are recorded in each case including age, gender, country of origin, travel history in the six months preceding infection and, if applicable, the identified source person. For 72 out of 204 reported patients, the diagnostic tests had been conducted by the Public Health Laboratory and of 63/72 patients, sera had been stored. In 54/63 samples, hepatitis B-virus-DNA could be amplified and sequenced.

Isolation, amplification, and sequencing

A genomic region of HBsAg that defines the different subtypes of the virus, was amplified and sequenced [3,4,5]. DNA was isolated from serum samples using standard techniques [6]. In each case the first HBsAg-positive serum sample obtained after infection, was chosen for amplification by PCR using primers ACPR and S3 (ACPR, nucleotide 56-85, sense: 5'-CCT.GCT.GGT.GGC.TCC.AGT.CCC.GGA.ACA.GTA-3'; S3, nucleotide 806-786, antisense 5'-TTG.GTA.ACA.GCG.GTA.TAA.AGG-3'). In those cases, in which the amount of PCR product was not sufficient for further analyses, a semi-nested PCR was performed using primers S1 (S1, nucleotide 459-479, sense: 5'-GTA.TGT.TGC.CCG.TTT.GTC.CTC-3') and S3. Standard PCR conditions were used taking stringent precautions against contamination as described previously [7]. Briefly, the PCR products were purified with Qiaquick columns (Qiagen, Germany). Both strands were sequenced with the primers S1, S2 (nucleotide

687-668, sense: 5'-GGC.ACT.AGT.AAA. CTG.AGC.CA-3') and S3. Sequence reactions were performed on a Vistra Labstation (Pharmacia Amersham, Roozendaal, The Netherlands) using dye terminator chemistry and analyzed on an Applied 373 automated sequencer (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Both strands were sequenced and analyzed using Geneworks software (Oxford Molecular, Oxford, United Kingdom).

Phylogenetic analysis

Hepatitis B-virus nucleotide (nt) sequences encompassing a 252-nt region of the surface gene (S gene) were obtained from 54 individuals. Nucleotide alignments were subjected to phylogenetic tree analysis, using the neighbor-joining method as implemented in the MEGA program [8]. Nucleotide distances were calculated according to the Kimura-2-parameter model [9], which takes into account different transition/transversion rates. The bootstrap option in MEGA (100 replicates) was used to determine the reliability of the clusters in the neighbor-joining trees. The neighbor-joining phylogenetic tree was compared in each case with a phylogenetic tree based on maximum likelihood calculations by using DNAML.exe as implemented in the PHYLIP software package [10] and by using the PUZZLE program [11,12].

Genetic distances for the hepatitis B-virus nucleotide sequences obtained from men having sex with men and injecting drug users were calculated by pair-wise comparisons according to the Kimura-2-parameter nucleotide substitution model.

Nucleotide sequence accession numbers

The nucleotide sequence data reported in this paper have been deposited in the GenBank sequence database under accession no. AY048597 to AY048650. The reference types used were obtained from the GeneBank: genotype A, substrain ayw1 (A_ayw1) = X75669 [3]; A_adw2=X75666 [3]; B_ayw1=X75660 [3]; C_ayr=X75667 [4]; C_adrq-=X75656 [13]; C_adrq+=X75792 [4]; D_ayw2=X75662 [4]; D_Costa Rica=U91832 [14]; D_ayw3=X75668 [3]; E_ayw4=X75657 [13]; F_adw4q-=X75658 [13].

Results

Participants

Table 1 presents the distribution according to probable transmission of the 54 index cases with acute hepatitis B in whose sera hepatitis B-virus-DNA was amplified and sequenced, in relation to the 204 notified cases (54/204=26,5%). The transmissions in these 54 persons were classified as follows: 19 men having sex

with men, 13 heterosexually transmitted, 10 injecting drug users, 2 household, 4 health care related, and 6 unknown transmissions. Patients with household- and unknown transmission were younger.

Table 1

Representation of risk groups in the study population (N=54) from 1992 to 1997 as classified by probable mode of transmission, based on interviews by a trained public health nurse in relation to the total number of primary index cases reported in this period.

Probable mode of transmission	Reported index cases	Number of strains sequenced (% of reported cases)		Male/ female (N=54)	Median age (N=54)	Age range (N=54)
MSM	71	19	(27)	19/0	29	20-36
HET	64	13	(22)	5/8	31	19-65
IDU	16	10	(56)	4/6	30.5	18-42
HOU	11	2	(18)	2/0	7	3-11
HCR	6	4	(67)	2/2	24.5	10-71
UNK	36	6	(17)	3/3	12.5	1-50
Total	**204**	**54**	**(27)**	**35/19**	**36**	**1-71**

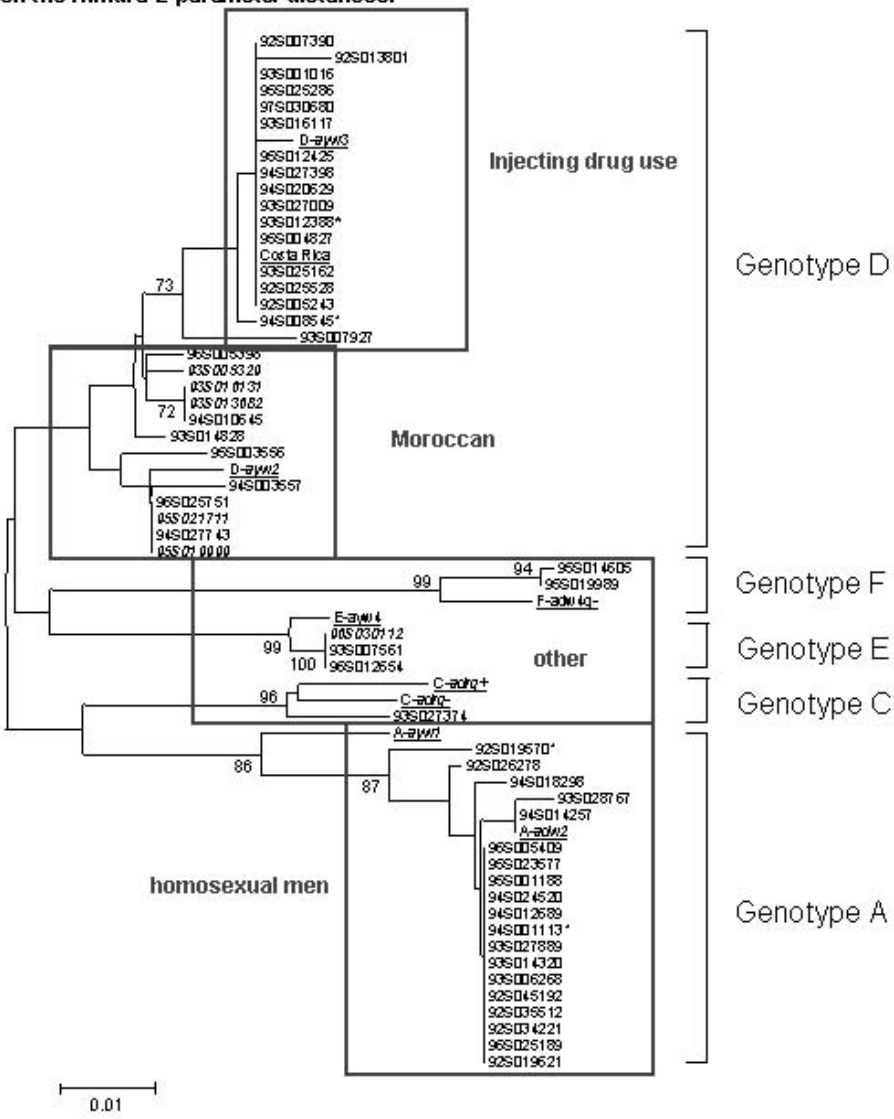
MSM risk behavior: men having sex with men (i.e. unprotected male homosexual contact)
HET risk behavior: unprotected heterosexual contact with new or multiple partners
IDU risk behavior: injecting drug use
HOU household contact who is hepatitis B carrier (no other risks identified)
HCR health care-related: invasive procedure (no other risks identified)
UNK unknown transmission

Sequence analysis

The hepatitis B-virus sequences are displayed in a phylogenetic tree constructed by the neighbor-joining method (Figure 1), which includes reference sequences for the distinct genotypes of hepatitis B-virus. The strains clustered in five genotypes: A, C, D, E and F. In hepatitis B-virus genotype A, there were 19 strains corresponding to reference serotype *adw2*. In hepatitis B-virus genotype D, two clusters could be identified, one with 17 strains corresponding to serotype *ayw3*, and one with 12 strains corresponding to serotype *ayw2*. Among the remaining strains, a high genetic variability was found, with isolates classified as belonging to hepatitis B-virus genotypes F, E and C and corresponding to serotypes *adw4q-*, *ayw4*, and *adrq-* respectively.

The phylogenetic tree based on maximum likelihood calculations gave similar results (data not shown).
The strains in the genotype A cluster, were similar in their diversity to the strains in the first genotype D cluster, serotype *ayw3*. The viruses in the genotype A cluster, differ from one another by 0% to 2.4%, with a mean genetic nucleotide distance of 0.4%, whereas viruses in the first genotype D cluster, differ by 0% to 2.4% with a mean distance of 0.37%. The strains in the second cluster in genotype D, serotype *ayw2*, differed by 0.8 % to 1.2%.

Figure 1. The neighbor-joining phylogenetic tree of 54 nucleotide sequences (S gene, surface antigen, 252 nt) of the hepatitis B virus obtained from individuals in Amsterdam, based on the Kimura-2-parameter distances.



Representative sequences for each genetic subtype obtained from the Genbank were included as references (Accession numbers: genotype A_ayw1= X75669; A_adw2=X75666; B_ayw1=X75660; C_ayr=X75667; C_adrq-=X75656; C_adrq+=X75792; D_ayw2=X75662; D_ayw3=X75668; E_ayw4=X75657; F=X75661;HB_CR=U91832). The two-digit numbers in the figure represent the bootstrap values obtained by generating 100 trees. Only bootstrap values of 50 and higher are shown. The sample numbers consist of year of diagnosis, S, and individual sample number. Sample numbers of cases classified as unknown are in *italics*; those of cases for which risk analysis gave a probable mode of transmission unrelated to the majority of the cluster are followed by an asterix*.

Epidemiological data related to clustering of strains

Of the 19 individuals with hepatitis B-virus of genotype A, 17 were men having sex with men. This cluster is referred to as the “cluster homosexual men”. Three individuals in this cluster did not have a Western European country of origin, one came from Brazil, one from Canada, one from Surinam. Of the 19 sequences, 14 were identical: 13 men having sex with men, one female classified as heterosexual transmission. In the five remaining strains of the “cluster homosexual men”, more diversity was identified. Four strains, which differed 0.4% to 0.8% from the 14 identical strains, were found in men having sex with men who were, based on travel history, probably infected abroad. The fifth strain was found in a man of Surinamese origin classified as heterosexual transmission. He was most likely infected through heterosexual contact with a Surinamese woman with chronic infection, whose virus had a sequence identical to his (data not shown). His strain differs 1,61% from the 14 identical strains. The female patient classified as heterosexually transmitted among the 14 men with identical strains, was a Dutch prostitute, whose male partner had acute hepatitis B six months earlier; no data on his risk behavior nor serum was available.

Of the 17 individuals with a hepatitis B-virus strain in the first subcluster in genotype D, corresponding to serotype *ayw3*, 9 were injecting drug users. We therefore refer to this as the “cluster drug users”. All had a Western European country of origin. Of the 17 viral sequences, 14 were identical. The 14 individuals with this strain were 7 injecting drug users, 6 heterosexual transmission, 1 health care related transmission. The source of the health care related case was identified based on sequence analysis as a former injecting drug user with chronic infection, co-admitted on the same psychiatric ward [Leentvaar-Kuipers, 1995]. In 5 of the 6 heterosexually transmitted cases, contact with a drug-using sexual partner was confirmed. The three divergent strains in the “cluster drug users” were found in two injecting drug users, differences 0.8% and 2.02%, and 1 heterosexual transmission. Based on her travel history, this person was probably infected abroad. Her strain differed 0.4% from the 14 identical “drug users strains”.

Of the 12 strains in the second subcluster in genotype D, corresponding to serotype *adw2*, nine were found in individuals of Moroccan origin, two had a country of origin in the America’s, one was Dutch. In this so-called Moroccan-cluster, one was heterosexually transmitted, a Moroccan male with a female Moroccan HBsAg-positive sexual partner; two were household transmissions, both Moroccan children with a HBsAg-positive family member; three were health care related, i.e. Moroccans with no risk factor other than a percutaneous procedure in a Moroccan health care setting; three were classified as unknown transmission, since they had no HBsAg-positive family member or other known risk factors. The three persons in this cluster with non-Moroccan origin included a female

prostitute from Middle America, classified as heterosexual transmission, whose strain was identical with one of the three Moroccan “unknown strains”. It was also identical to the strain of the second non-Moroccan person in this cluster: a female of South American origin. This woman, classified as unknown transmission, had spent nine months preceding her infection in Venezuela. Her family members in The Netherlands were all tested, and appeared to be HBsAg-negative. The history on heterosexual risk behavior remained inconclusive. The third non-Moroccan person in the Moroccan cluster is a 12-year-old Dutch male, also classified as unknown transmission. The majority of his class- and playmates were of Moroccan origin.
Genotype F was found in two Dutch men having sex with men. Both were infected in 1995, had not traveled in the months preceding their illness and had no sexual relationship, or shared sexual partner.
Of the three strains in genotype E, 2 were heterosexual transmission, 1 was unknown transmission. One heterosexually transmitted female was infected through heterosexual contact with a Nigerian man, with chronic infection. The sequence of his isolate was identical to hers (data not shown). The second HET was a female prostitute of South American origin, who arrived in the Netherlands three months before the first day of illness. For the third case, a Dutch male classified as unknown transmission, no possible source could be identified.
The individual with the genotype C strain was classified as heterosexual transmission because this female Dutch travel guide had heterosexual risk behavior in Southeast Asia in the months preceding her infection.

Discussion

This is the first epidemiological study of community acquired acute hepatitis B using phylogenetic analysis. Previous studies have analyzed strains originating from individuals with chronic infection with hepatitis B virus, [3,4,16,17] reflecting the epidemiological situation of vertical transmission in endemic countries or in individuals originating from such countries. Sequencing is used with success to link cases in an outbreak setting [18,19,20,21,22]. In this study we analyzed new incident cases in a metropolitan area. We identified three distinct genotypes of the hepatitis B-virus in Amsterdam in different risk groups: a genotype A cluster, related to hepatitis B transmission between men who have sex with men, with a predominant “homosexual men strain”; a genotype D cluster, related to drug use or heterosexual contact with drug users, with a predominant “drug users strain”, and a third cluster, also in genotype D, related to the Moroccan population in Amsterdam, the “Moroccan cluster”. The Moroccan strains correspond with the

hepatitis B-virus subtypes found in the Mediterranean area [17,23].
This study shows that phylogenetic analysis has important value for public health programs. Firstly, it confirms that the history-taking of experienced public health nurses brings to light risk behavior that corresponds well with the clustering of strains.
Secondly, these data support other observations from European sequencing studies with HIV [24] and HAV [25], that there is very little spread of sexually transmissible virus from the homosexual to the heterosexual population. Only 1/14 identical “homosexual men strains” was found in a heterosexual. A different “homosexual men strain”, genotype F, was found twice in 1995 in male homosexuals and never again. Given the transmission dynamics among men having sex with men, an incidentally imported hepatitis B-virus strain is unlikely to become endemic in The Netherlands. In contrast to other continents, Europe seems to have rather distinct circuits of sexually active populations.
Thirdly, phylogenetic analysis points to considerable hepatitis B transmission from injecting drug users to the heterosexually active population, as was observed with HIV-1 in a sequence study conducted in Amsterdam [26]. Proper history-taking to detect drug-related contacts of the heterosexual population is appropriate. In 5/6 as heterosexual classified transmissions, in whom a “drug users type” virus was isolated, a (heterosexual) contact with a drug user was confirmed. Programs directed at vaccinating drug users will have additional beneficial effect in reducing heterosexual transmission to the non drug using population. A project aimed at vaccinating high risk groups started in the Netherlands in 1998, with Amsterdam as the major pilot area. The coverage of vaccination among injecting drug users is disappointing [27]. This analysis suggests that the failure to reach all injecting drug users, will affect hepatitis B incidence in the broader heterosexual population. Since the project is also directed at vaccinating visitors of STD-clinics and visitors of prostitutes, including those of drug using prostitutes, the project might still reduce the spread of hepatitis B in the broader heterosexual population.
The fourth public health value of this study is its insight into the importance of transmission of hepatitis B virus from HBsAg-positive individuals either acquired while traveling to or by origin from hepatitis B-endemic countries. Six individuals were infected while traveling abroad, one possibly as a resident abroad.
The data suggests that three health care related cases, all in Moroccan individuals, were infected through transmission within the Moroccan community, without excluding nor confirming the possible effect of invasive health care intervention in Morocco. Also, both household transmissions took place within this community, and three of the six unknown cases were Moroccan individuals. No homosexual men or “drug users strains” were isolated in cases with unknown transmission.

The Health Council of The Netherlands has advised to vaccinate children with one or both parents originating from a hepatitis B-endemic country [28]. Our data suggest that this policy would contribute in preventing health care related, household and unknown transmitted cases.
In the public health setting, the value of sequencing assists little to trace individual sources for individual cases. In our set of data it helped in pinpointing the source in one health care related case and two heterosexually transmitted cases. However, for most individual cases the sequencing can not prove who the actual source person is. The virus isolated from the Dutch boy with contacts of Moroccan origin, belonged to the Moroccan cluster. Since hepatitis B virus transmission in schools has been documented [29], this finding suggests a possible source in the Moroccan community. A vaccination policy as proposed by the Health Council will prevent similar transmissions.
Our findings have important consequences for the prevention of hepatitis B in countries where universal childhood hepatitis B vaccination is not yet or only recently implemented. At present, vaccination campaigns in these countries are directed at high-risk groups for acute infection (men having sex with men, injecting drug use and heterosexual transmission). Our data indicate that besides these well known transmission routes, there is considerable transmission within and from people originating from hepatitis B endemic countries. The existing antenatal screening and neonatal vaccination program identifies pregnant carriers, of whom in Amsterdam 97% are of foreign origin [30]. Male or non-pregnant females will only be identified if they are in the immediate family or household of an HBsAg-positive expectant mother or new incident case. We suggest to further investigate the contribution of unknown HBsAg positive sources that are missed by screening only pregnant mothers in this population.
Our study population represents only 26% of all cases of acute hepatitis B infection in Amsterdam. There is an over representation of injecting drug users and health care related cases. We therefore can not estimate the contribution of the various risk groups in the total hepatitis B transmission. However, the relative importance of drug users in heterosexual transmission, and the relative importance of chronic infected individuals from hepatitis B endemic countries in household and unknown transmission, is not influenced by this skewed selection of samples. The existence of the Moroccan cluster, and the various incidental exotic genotypes found in this analysis, show that, even with complete coverage by the existing vaccination programs, transmission of hepatitis B virus will continue to occur. This will in part be prevented by the policy as proposed by the Health Council. Further screening of all newcomers from hepatitis B-endemic countries should be considered. Treatment should be offered to all thus identified chronic infected potential sources. Active source and contact tracing in the case

of each newly identified infection or chronic infected individual remains an essential strategy to prevent horizontal and sexual transmission. Without universal vaccination, public health policy to prevent spread of hepatitis B in the Netherlands must depend on a complete inventory of sequences from isolates representing all reported hepatitis B cases.

Conclusion

In this retrospective molecular epidemiological study, we show that the 54 sequenced and analyzed viruses could be grouped directly or indirectly into one of three risk groups: homosexual, drug-related or imported. The results of sequencing have important value for public health intervention. Our analysis provides evidence that hepatitis B virus transmission from injecting drug users to the non-drug-using heterosexually active population is frequent. Transmission from the homosexual population to the heterosexual population is limited. Transmission from men having sex with men to injecting drug users and vice versa is not seen. There is considerable spread of hepatitis B virus from chronic infected people originating from hepatitis B endemic countries. The present vaccination policy in The Netherlands consists of an active approach towards the neonates of carrier mothers, household contacts of incidentally identified carriers, individual targeted-risk persons, such as travelers, men having sex with men, injecting drug users, prostitutes and their clients, and other heterosexually active individuals. This policy is not sufficient to eliminate transmission of hepatitis B virus from chronic infected individuals from endemic areas. We conclude that the policy, as suggested by the Health Council, to vaccinate all children with one or both parents originating from hepatitis B endemic countries, gives an important contribution to eliminating hepatitis B in The Netherlands. Besides this proposed strategy we suggest to place more emphasis on tracing and protecting susceptible individuals at high risk for contracting an imported strain, i.e. sexual and household contacts of (unknown) chronic infected individuals from hepatitis B -endemic countries.

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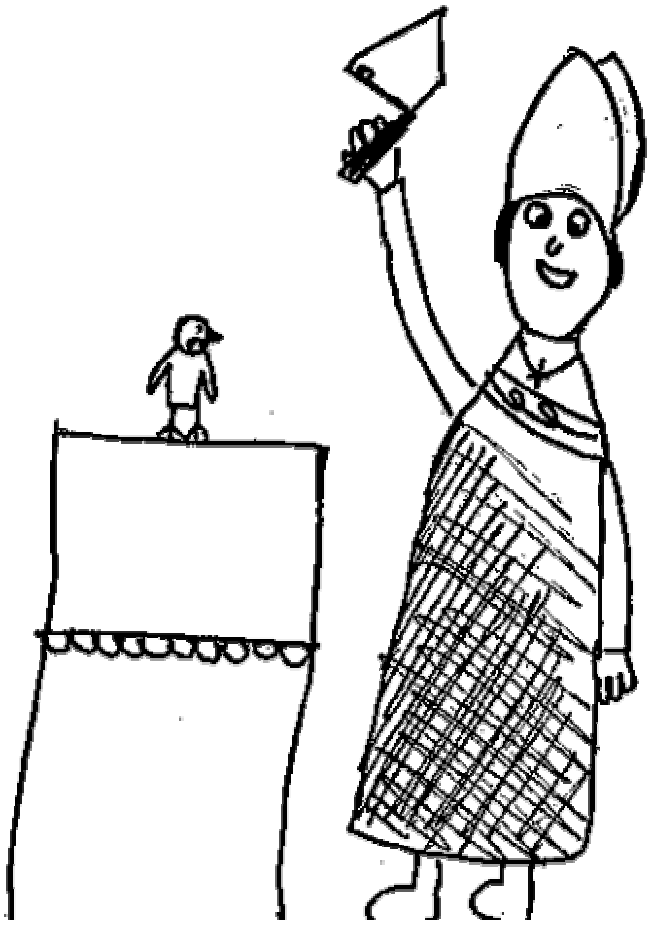

Early civilisations near the region of Mesopotamia regarded the liver as the seat of life because it appeared to be the central collection point for blood. Detailed examination of the liver of sacrificed animals was used by Mesopotamian doctors for forecasting illnesses or for planning military campaigns. This practice of divination extended in to the fifth century B.C. Thus, it is not surprising that liver disease and jaundice were relatively well known to the Babylonians and other peoples of antiquity. Both the Babylonian Talmud and the writings of Hippocrates allude to jaundice as a symptom complex.
F.Blaine Hollinger, in Fields Virology pp 2739

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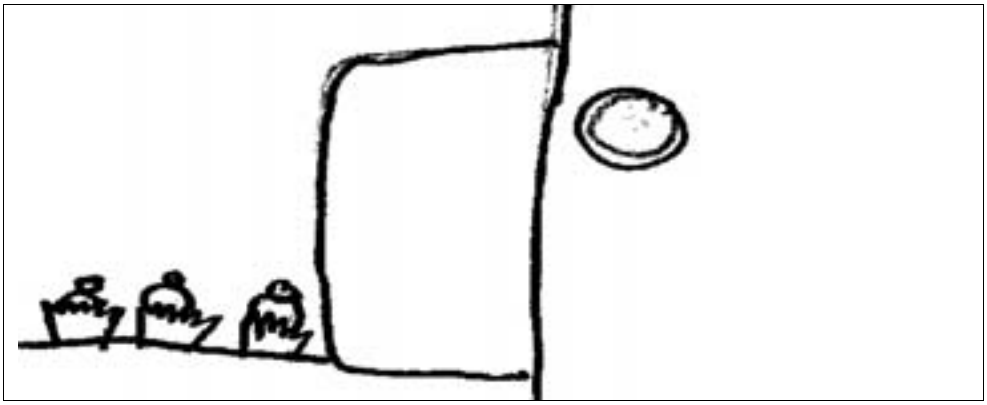
Chapter 6

HAV immunity and seroconversion in contacts of acute hepatitis A patients in Amsterdam, 1996-2000; *evaluation of current prevention policy*

Gerard J.B. Sonder (1,2), Jim E. van Steenbergen, (1,3), Lian P.M.J. Bovee, (1), Paul G.H. Peerbooms, (4), Roel A. Coutinho, (1,5), and Anneke van den Hoek, (1)



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1. GG&GD (Municipal Health Service), Dept. of Infectious Diseases, Amsterdam, The Netherlands (NL)
2. LCR (National Co-ordination Center for Travelers Health Advice), Amsterdam, NL.
3. LCI (National Co-ordination Communicable Disease Control), Utrecht, NL.
4. GG&GD, Public Health Laboratory, Amsterdam, NL.
5. Academic Medical Center, University of Amsterdam, Dept. of Human Retrovirology, Amsterdam, NL.

Abstract

Objectives- To evaluate the current hepatitis A control policy of householdcontacts of acute hepatitis A patients between 1996 and 2000 inclusive.

Methods- The characteristics and serological outcome of contacts were analyzed. All susceptible contacts were invited for retesting 6 weeks after receiving immune globulin (IG).

Results- Of 569 patients, 1242 contacts were analyzed. Over 50% (672) were HAV immune. Of the remaining 570, 161 (28.2%) had a concurrent infection, of whom 86/161 were symptomatic. The remaining 409 susceptible contacts received IG, 186/409 (45%) came back after 6 weeks, of whom 64 (34%) were infected, but only 12 with symptoms. No tertiary cases were reported.

Conclusions- IG does not protect all contacts from HAV infection but rather attenuates symptoms. IG effectively seems to reduce further HAV transmission.

Introduction

Hepatitis A is an acute liver disease caused by the hepatitis A virus (HAV), which is transmitted by the feco-oral route. The disease is rarely symptomatic in children under 5, but morbidity and mortality can be high in adults. Its prevalence is strongly related to economic conditions: in less developed countries the disease occurs widely among children and, as a result, most adults are immune. In more developed countries, more adult cases and clinical infections are seen. In the Netherlands, as in most western countries, the seroprevalence of anti-HAV antibodies declined sharply in people born after World War II [1], making a majority of the population susceptible.
In Amsterdam, the incidence of hepatitis A follows a largely seasonal pattern, with peak incidences in August and September due to import of the virus by children of migrant-worker families (mainly from Turkey and Morocco) who have spent summer holidays in the country of parental origin [2], Hepatitis A also causes year-round micro-epidemics among homosexual men [3,4], but sequencing of the viruses suggests that different subgenotypes circulate in different risk-groups [5].
In the Netherlands, a diagnosis of hepatitis A is notifiable and must be reported to the Municipal Health Service (MHS). To prevent secondary cases, household contacts of each primary patient are identified and given advice on hygienic precautions, as well as passive immunization with IG if found susceptible.
In this study we evaluated the serological results of testing household contacts of acute hepatitis A reported 1996-2000 to determine the proportion of those immune at presentation and the predicting factors for such immunity. Furthermore we analyzed the follow-up of the susceptible contacts to determine the incidence of symptomatic and asymptomatic HAV infection.

Participants and methods

Classification
This study was based on all hepatitis A cases reported to the Department of Infectious Diseases of the Municipal Health Service (MHS) in Amsterdam between 1 January 1996 and 31 December 2000. After a case is reported, a history is taken to find the most likely route of HAV transmission. According to these routes, we classified patients hierarchically into 5 'transmission' groups. If homosexual activity had occurred in the previous 6 weeks, this was considered the most likely cause of infection. If no homosexual activity had occurred, but there was a hepatitis A case in the immediate environment, this case would be the

most likely cause of infection. If travel to a highly HAV-endemic country had occurred in the previous 6 weeks, this was the most likely cause. Primary school students who did not travel, were most likely to have been infected by an asymptomatic peer at school. Patients with no likely cause of disease were in the 'unknown' group. Household contacts are all people living in the same house and sharing the same toilet as the index case, as well as people who take care of a child with hepatitis A and sexual partners of an index patient.

Interventions

All household contacts are invited to receive MHS advice on hygienic precautions; were offered serologic testing (total anti-HAV antibodies) and immunization with immune globuline (IG) within 14 days after the onset of disease in the index-patient. The first day of disease in an index-patient was defined as the first day of jaundice. Since people born and raised in highly HAV-endemic countries are often immune, they are not given IG until the HAV antibody test results are available (usually within 1 day). Children under 10 years of age who test positive for total anti-HAV are tested also for IgM antibodies to ascertain whether they have a recent infection. Contacts over 10 years old are tested for IgM only if they describe symptoms indicative of acute hepatitis A.

Follow up

To detect infections occurring within 6 weeks after passive immunization, susceptibles are invited for retesting. Those who then test positive for total anti-HAV are also tested for anti-HAV IgM to exclude possible false-positive tests caused by IG administration. Only people with a positive IgM antibody test were considered to have seroconverted and acquired a recent hepatitis A infection. We detected antibodies against hepatitis A virus using a competitive enzyme immunoassay for total antibodies and an antibody-capture enzyme immunoassay for the detection of IgM-antibodies (HAVAB and HAVAB-M, Abbott Diagnostic Division, Wiesbaden, Germany). A solid phase version of both tests were used until April 1998, then replaced by a microparticle version (AXSYM®, Abbott Diagnostic Division). The sensitivity of these tests is 99.7%; the specificity is 99.0%. The incubation period of hepatitis A varies between 14 to 50 days. Therefore we classify persons with a disease onset within 14 days of disease onset in the index patient as concurrent primary or co-primary cases. Persons with disease onset between 15-50 days after disease onset in the index patient were considered secondary cases [6].

For this, data from all hepatitis A patients and their contacts were extracted from the electronic database of the Department of Infectious Diseases: date of birth, gender, symptoms of disease, date of onset of disease, risk factors in the incuba-

tion period, birth-country, parents' birth-country, the date of passive immunization and dates and results of blood tests (total HAV antibodies and IgM antibodies). For people older than 15 years, country of origin was defined as the country of birth. For people 15 years or younger, country of origin was defined as the birth-country of their parent(s).

Statistical analysis
Chi-square test or Students' t-test was used where appropriate, to compare characteristics between different groups. For calculating risk factors for different outcomes, SPSS logistic regression was used to obtain univariate and multivariate odds ratio's (ORs) and 95% confidence intervals (CIs). In multivariate modeling, all factors with a p-value < 0.10 were included.

Results

Reported cases (Figure 1)
Between 1 January 1996 and 31 December 2000, 569 patients with IgM-confirmed acute hepatitis A (index patients) were reported to the MHS in Amsterdam (Table 1). In 151 (26.5%) homosexual activity was the most likely transmission route; 66 (11.6%) had close contact with a symptomatic patient; in 158 (27.8%) travel to an endemic country was the most likely transmission route; 74 (13.0%) were primary school students with no cases in their immediate environment and without travel history. For the remaining 120 (21.1%) no obvious source of infection was found.
Most homosexually infected men were born in Western countries (89%). Most otherwise infected patients were 15 years or younger (71%) and of Moroccan origin (59%).

Table 1
Characteristics of all to the MHS in Amsterdam, Netherlands (NL) reported cases of acute hepatitis A between 1 January 1996 and 31 December 2000, by sexual orientation.

Characteristics of Index patients	***Homosexual transmission***	***Other than homosexual transmission***
Total	151 (26.5%)	418 (73.5%)
Sex		
Female		216 (51.7%)
Male	151(100%)	202 (48.3%)
Mean age in years (min-max)		
Female		14.0 (1-77)
Male	35.1 (19-72)	15.7 (2-63)
Country of origin* for persons > 15y		
NL and other Western countries	134 (88.7%)	107 (85.6%)
Morocco		4 (3.2%)
Turkey		1 (0.8%)
Other non-Western	14 (9.3%)	10 (8.0%)
Unknown	3 (2.0%)	3 (2.4%)
Country of origin* for persons < 16y		
NL and other Western countries		26(8.9%)
Morocco		172 (58.7%)
Turkey		37 (12.6%)
Other non-Western		22 (7.5%)
Country of birth=NL, parents' birth country unknown		35 (11.9%)
Unknown		1 (0.3%)
Age group		
0-15 y		298 (71.3%)
16-35 y	92 (60.9%)	74 (17.7%)
> 35y	59 (39.1%)	46 (11.0%)
Number of household contacts		
no contacts	88(58.3%)	45(10.8%)
1 contact	56(37.1%)	38 (9.1%)
2-3 contacts	6(4.0%)	113(27.0%)
>3 contacts	1(0.7%)	222(53.1%)

*For people older than 15 years, country of origin is defined as the country of birth. For people 15 years or younger, country of origin is the country of their birth unless that country is The Netherlands; then country of origin is the country of the parents'.

It appears that the contagious nature of jaundice was first implied in the Eighth century AD, in letters from Pope Zacharias to St Boniface, Archbishop of Mainz in 751.

Arie Jeremy Zuckerman. The History of Viral Hepatitis from Antiquity to the Present. In: Viral hepatitis: laboratory and clinical science. Friedrich Deinhardt and Jean Deinhardt (Eds). 1983

De his qui regio morbo vexantur inquisisti, sive hominis, sive equi sint, quid faciendum sit de illis. Si homines ex nativitate, aut genere, hajus morbi sunt, hi extra civitatem commanere debebunt, ad eleemosynam vero accipiendam a populo non devitari. Si autem contigerit magnum vel parvum non nativitate , sed superveniente aegritudine vexari, non est projiciendus, sed, si possibile est, curandus

J.-P. Migne. Patrologiae, cursus completus, 1863. Epistola XIII, Zachariae Papae ad Bonifacium Archiepiscopum, 951

Immune contacts (Figure 1)

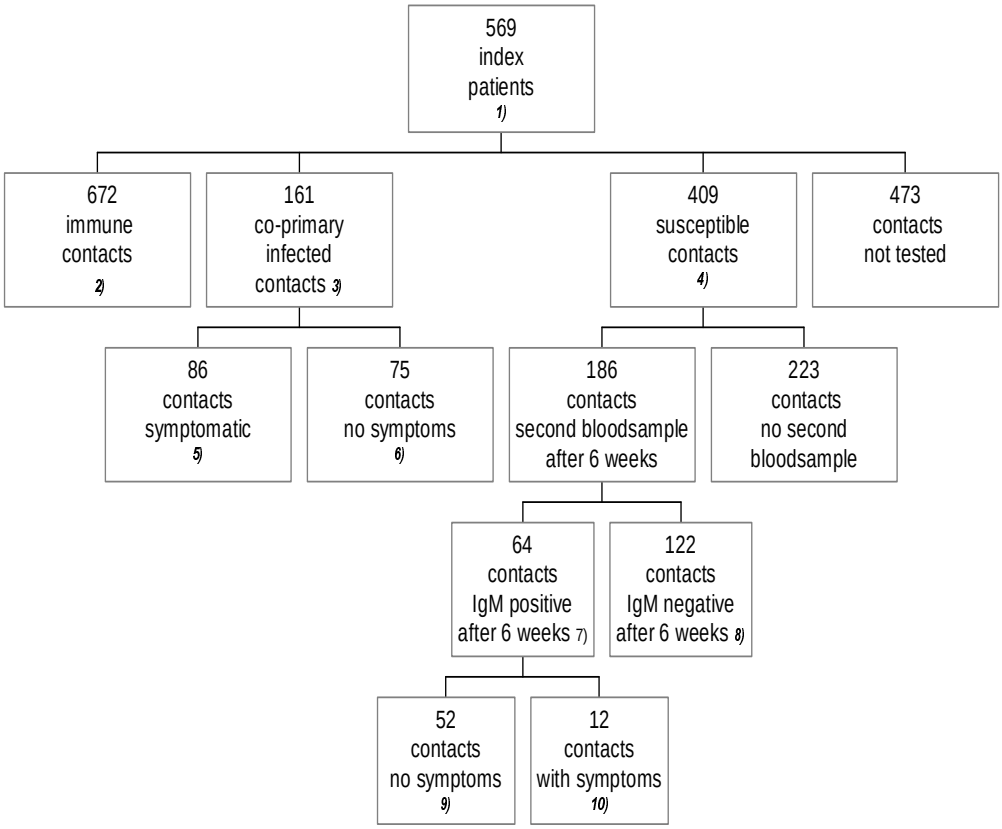
A total of 1715 household contacts were identified, each index patient having an average of 3 contacts (range 0-16). Of these 1715 contacts, 473 were excluded because blood samples were not taken, or were taken more than 14 days after disease onset in the index patient.

The characteristics of the 1242 remaining contacts are presented in table 2. Of these, 672 (54%) were immune at presentation. Dividing total contacts by age group, 359 were aged 10 or younger, of whom 35 (10%) were immune.

The included and excluded groups did not differ in gender, number of contacts, or most likely source of infection. However, the median age of the excluded contacts was significantly lower (15 years, range 0-64) than that of the included group (20 years, range 0-77) ($p < 0.001$). Significantly more contacts were not included if the 'country of origin' was 'Other, non-Western' ($p = 0.03$).

Figure 1

Outcome of 1715 contacts of 569 acute hepatitis A patients in Amsterdam, The Netherlands 1996-2000.



1) All notified to MHS with jaundice and anti-HAV IgM positive
2) No symptoms, anti-HAV total positive, if < 10 years old IgM negative
3) All IgM positive
4) No symptoms and anti-HAV total negative
5) With symptoms and IgM positive
6) Without any symptoms and IgM positive. Because contacts older than 10 years without symptoms are not tested, everybody in this group is 10 years or younger.
7) Anti-HAV total negative in the first blood-sample, total anti-HAV positive AND anti-HAV IgM positive in the sample 6 weeks later.
8) Anti-HAV total negative in the first blood-sample and in the sample 6 weeks later
9) IgM positive but no symptoms of any disease
10) IgM positive and symptoms, not always jaundice

Predictors for immunity (Table 2)

In univariate analysis, contact age, transmission group of index-patient and contact gender, number of household contacts and country of origin, were significantly associated with immunity at presentation. All these factors were included in multivariate analysis. Older age, the travel or school transmission groups, four or more household contacts, and origin in highly endemic countries were independently positively associated with immunity. Contacts of patients in the homosexual transmission group were significantly less likely to be immune at presentation than contacts in other groups. Significantly more people originating from HAV-endemic countries were immune at presentation than people originating from Western countries.

Table 2

Prevalence of hepatitis A antibodies in blood samples, taken from contacts within 14 days after onset of hepatitis A in the index patient, by characteristics, Amsterdam, the Netherlands 1996-2000.

Characteristics of contacts	***Total (%)***	***Anti-HAV+***	***OR (95% CI)*** univariate	***OR (95% CI)*** multivariate
Total	1242	672(54.1%)		
Age groups				
0-5 y	160	5(3.1%)+	1	1
6-10 y	199	30(15.1%)+	5.5(2.1-14.5)***	5.0(1.9-13.4)**
11-15 y	170	82(48.2%)	28.9(11.2-73.9)***	26.0(10.0-67.7)***
>15y	713	555(77.8%)	108.9(43.9-269.9)***	1587.0(520.3-4840.5)***
Transmission group index				
unknown	297	140(47.1%)	1	1
travel	492	306(62.2%)	1.8(1.4-2.5)***	2.4(1.5-3.8)***
homosexual activity	51	13(25.5%)	0.4(0.2-0.8)**	0.3(0.1-0.9)**
school	232	137(59.1%)	1.6(1.1-2.3)**	2.2(1.3-3.7)**
case in immediate environment	170	76(44.7%)	0.9(0.6-1.3)	1.1(0.6-2.0)ns
Gender				
Male	618	304(49.2%)	1	1
Female	624	368(59.0%)	1.5(1.2-1.9)***	1.0(0.7-1.4)ns
Number of household contacts				
1 contact	69	21(30.4%)	1	1
2-3 contacts	223	98(43.9%)	1.8(1.0-3.2)*	0.6(0.3-1.6)ns
>3 contacts	950	553(58.2%)	3.2(1.9-5.4)***	2.2(1.0-4.9)*
Country of origin++				
NL and other Western countries	310	120(38.7%)	1	1
Country of birth=NL, parents' birth country unknown	53	8(15.1%)	0.3(0.1-0.6)**	30.9(9.8-96.8)***
Turkey	144	85(59.0%)	2.3(1.5-3.4)***	22.2(9.7-50.8)***
Morocco	647	398(61.5%)	2.5(1.9-3.3)***	39.8(19.8-80.2)***
Other non-Western	88	61(69.3%)	3.6(2.2-5.9)***	14.7(6.4-33.6)***

\+ All children under 10 years old were IgM negative.

++ For people older than 15 years, country of origin is defined as the country of birth. For people 15 years or younger, country of origin is the country of their birth unless that country is The Netherlands; then country of origin is the country of the parents'.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Co-primary cases (Figure 1). Onset of hepatitis A infection in contacts within 14 days after onset in index patient
Of the 570 non-immune contacts, 161 (28.2%) tested IgM positive at their first bloodtest. These cases are considered co-primary infections. Of all co-primary infections, 86/161 (53%) were symptomatic; 127 (79%) of these infections were in children of 10 years or younger of whom 52 (41%) were symptomatic. No asymptomatic infections in contacts older than 10 years were found because these contacts were not tested for IgM antibodies.

Secondary cases (Figure 1). Seroconversion in susceptible contacts (Table 3):
Of the 409 susceptible contacts, 186 (45.0%) returned for a second bloodtest 6 weeks later. Of these, 64/186 (34%) were IgM positive so they had acquired a secondary infection. Twelve of them (19%) were symptomatic cases. Age was not related with symptomatic hepatitis A infection (data not shown).
Between the groups that did and did not return, no significant differences were found for age group, mean age, gender or number of contacts. However, the proportion of people who returned did vary with national background ($p < 0.05$). Of the people from Western countries 39% came back; from 'children born in the Netherlands, origin parents unknown' 31% came back, from Morocco 55%; from Turkey 38% and from 'other non-Western countries' 52%. Tertiary cases were not identified nor reported to the MHS later.

Table 3
Seroconversion in susceptible contacts of patients with acute hepatitis A, by characteristics, in Amsterdam, The Netherlands 1996 – 2000.

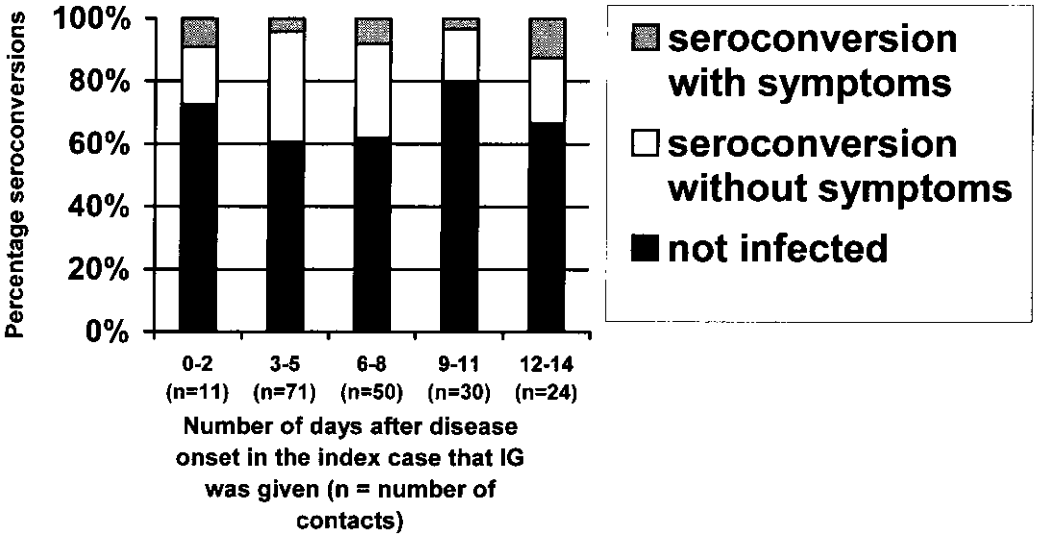
Characteristics of contacts	Total (%)	seroconversion univariate	OR (95%CI)
Susceptible contacts:	186	64(34.4%)	
Age group			
0-5 y	33	11(33.3%)	1.2 (0.5-3.1)
6-10 y	59	20(33.9%)	1.3(0.6-2.8)
11-15 y	35	16(45.7%)	2.1(0.9-5.0)
>15 y	59	17(28.8%)	1.00
Transmission group index			
travel	66	21(31.8%)	1
homosexual activity	17	6(35.3%)	1.2(0.4-3.6)
unknown	55	15(27.3%)	0.8(0.4-1.8)
school	31	14(45.2%)	1.8(0.7-4.2)
case in immediate environment	17	8(47.1%)	1.9(0.6-5.6)
Gender			
Male	103	36(35.0%)	1.1(0.6-1.9)
Female	83	28(33.7%)	1
Number of household contacts			
1 contact	20	6(30.0%)	1
2-3 contacts	31	12(38.7%)	1.5(0.4-4.9)
>3 contacts	135	46(34.1%)	1.2(0.4-3.4)
Country of origin+			
NL and other Western countries	60	19(31.7%)	1
Country of birth=NL, parents' birth country unknown	8	3(37.5%)	1.3(0.3-6.0)
Turkey	17	3(17.6%)	0.5(0.1-1.8)
Morocco	90	37(41.4%)	1.5(0.8-3.0)
Other non-Western countries	11	2(18.2%)	0.5(0.9-2.4)

+ For people older than 15 years, country of origin is defined as the country of birth. For people 15 years or younger, country of origin is the country of their birth unless that country is The Netherlands; then country of origin is the country of the parents'.

In univariate analysis, no variables like contact, age, transmission group (of index-patient), gender, number of household contacts or country of origin, were significantly associated with secondary infection. As shown in Figure 2, there was no association between the time at which IG was given and the likelihood to seroconvert: OR 0.96 (0.83-1.04), nor was there an association between the time at which IG was given and the likelihood to contract a symptomatic disease: OR 0.96 (0.83-1.12).

Figure 2

Total of all susceptible contacts (n=186) of acute hepatitis A patients, to whom immune globuline (IG) was given, by day after disease onset in the index case, with outcome of disease in those contacts 6 weeks later.



Discussion

This study is an evaluation of the current policy in prevention of secondary transmission of hepatitis A in Amsterdam, based upon analysis of routinely collected data. The relatively high rate of 50% immunity in contacts may be explained by the high endemicity of HAV in their country of origin. Of the non-immune contacts 28% had a co-primary infection.
Of 186 susceptible contacts, despite administration of IG within 2 weeks after onset of disease in the index-patient, 34% developed a secondary infection. Only 6% developed a secondary clinical infection.

Index cases

In Amsterdam there are two main transmission-groups of hepatitis A: travelers to highly endemic countries (many of them are children originating from Morocco and Turkey) [2] and homosexual men. Molecular sequencing showed that there are two main separate groups: two different subgenotypes are identified in these two groups [7].

In Amsterdam, since 1998, we started an annual vaccination campaign for children under 16 years travelling to HAV endemic countries (mainly Turkey and Morocco), resulting in a coverage of 50% [8]. All other travelers to HAV endemic countries are also recommended vaccination.

The group of homosexual men is more difficult to protect: transmission occurs year-round and mainly from anonymous contacts. Because homosexual men are one of the two largest groups of acute hepatitis A cases in Amsterdam, we think all homosexual men in Amsterdam should receive vaccination against hepatitis A. In the Netherlands, in November 2002 a vaccination campaign started, offering homosexual men free vaccination against hepatitis B. An effort is made to offer this group, instead of a hepatitis B vaccine, a combined hepatitis A and B vaccine for a reduced price.

The last 4 years the incidence of notified cases of hepatitis A in Amsterdam seems to decrease from 200 in 1998 to 50 in 2002 [9]. Whether this is an ongoing trend still has to be seen.

Immunity of contacts

Of 1242 contacts, more than 50% were immune. The average age in the excluded group, without a blood sample, was significantly lower than in the age of the included group, because parents often object to blood sampling in children when it is not therapeutically necessary. As children are more likely than adults to be susceptible to HAV, our rate of immunity may be an overestimation.

Because we did not test for IgM in contacts > 10 years who were total anti-HAV positive, we may have classified some asymptomatic co-primary infected contacts > 10 years as immune, which may also have resulted in an overestimation of the immunity rate. However, as the large majority of acute hepatitis A in older people is symptomatic [10]. We do not expect this to be of major influence on the results.

Among contacts of index patients in the homosexual transmission group, immunity at presentation was relatively lower than in the other transmission groups, probably because most of these contacts were from countries with low HAV endemicity.

Co-primary cases

Of the susceptible contacts, 28 % were co-primary cases, seroconverting within 14 days of disease onset in the index; half had symptoms of hepatitis A. In Athens, 18,5 % co-primary cases were found among susceptible contacts of 113 children with hepatitis A; 3,5% were clinical cases [11]. All blood samples were taken within 7 days after disease onset in the index, and we infer that the rate of co-primary cases would have been higher than 18,5% if samples had been taken through 14 days. An Italian study, concerning household contacts of 380 hepatitis A patients in Naples [12], found 2,6% co-primary cases (9/219 susceptibles). Both studies focused on the contacts of sporadic index patients and did not mention the most likely cause of infection in the latter cases.

Secondary cases

Compared to other studies, we found a high (34%) secondary seroconversion rate in susceptible contacts despite IG treatment within 1-14 days. On the other hand, of all susceptible persons, only 12/409 (2.9%) secondarily contracted symptomatic hepatitis A, a finding in agreement with other studies [12]. Symptomatic people may be more likely to return after 6 weeks than people without symptoms, but excluding the symptomatic cases, we still found a seroconversion rate of 30% (52/174).

In only a few other studies was a second blood test performed to detect secondary asymptomatic infections. In the Italian study [12], 12/102 (11.8%) susceptible household contacts who received no IG nor vaccination seroconverted after 6 weeks (4/12 had symptoms), whereas 2/110 (1.8%) of contacts who received active immunization with hepatitis A vaccine, seroconverted asymptomatically. The Greek study [11] reported no seroconversions in 85/185 susceptible household contacts that came back 4 weeks after IG was given. Finally, a review of these two and four other studies [13] estimated the probability of secondary seroconversion of susceptible contacts in the presence of an HAV-infected index patient. The reviewers considered the proportion immune at presentation and the proportion of asymptomatic infections based on assumptions if the studies provided no measurements; they stratified for age, based on average age distributions in American families. The resulting rate of transmission to susceptible children under 12 years old was estimated 22% (95% CI: 12%-33%) and in susceptible adults 15% (CI: 9%-20%). We found a somewhat higher rate. A possible explanation for this difference may be the difference in transmission groups: both the Italian and the Greek study concerned only family contacts of sporadic index cases, in the Greek study all index cases were children. Even though in these studies the most likely route of transmission was not mentioned, it is unlikely that, like in Amsterdam, 28% of the index cases were infected by travel. In our study,

most contacts are contacts of index cases who traveled. Often they had traveled as companions of these index cases. Instead of a secondary case, some of them could as well have been co-primary cases with a long incubation period (see discussion paragraph 'limitations'). However, this suggestion is contradicted by the fact that we did not find significant differences in the various transmission groups with regard to secondary transmission (Table 3).

The high seroconversion rate despite IG adds evidence that IG prevents or attenuates symptoms but does not always prevents infection [14]. Without any intervention, the severity of hepatitis A and the proportion of infected people developing jaundice rises markedly with age: from 0% in children 0-3 year old to 80% in persons over 15 years old [10]. Of the co-primary infections among children under 10 years old in our study, 40% were symptomatic. Of all secondary infections despite IG in children, 19% were symptomatic, and in contacts over 15 years old 35% was symptomatic, much lower than the 80% one would expect [10]. Relatively more secondary than co-primary infections were asymptomatic, and for secondary infections age was not related with symptomatic infection. Both findings are probably due to the IG received by contacts.
When administered within 2 weeks of exposure to HAV, IG reportedly prevents more than 85% of clinical hepatitis A cases [15]. Administration of IG in day-care centers has stopped the spread of hepatitis A (clinical cases) [16]. If it does not prevent seroconversion but does reduce further transmission, a possible explanation is that IG diminishes HAV excretion.
Its efficacy is said to be greatest when IG is administered early in the incubation period [15]. In our study, the interval between administration of IG and disease onset in the index patient appeared unrelated to the chance to seroconvert. Also, no relation was found between this interval and the likelihood of developing a symptomatic disease; however, our number of symptomatic cases was small.
So far, contacts of acute hepatitis A patients in Amsterdam are protected from infection by administration of IG as soon as possible after the index case is notified. Recently, the hepatitis A vaccine used for pre-exposure prophylaxis in travelers to HAV-endemic countries, is also recommended instead of IG for post-exposure prophylaxis in our national guidelines [17]. Vaccination may not always be fast enough to prevent clinically overt disease, especially in people over 40 years of age or obese, who have a slower immune response to hepatitis A vaccination [18] or in persons vaccinated more than 7 days after disease onset in the index case [19]. Therefore vaccine is recommended for all healthy contacts under 30 years old. For contacts between 30 and 50 years, it is recommended only if administered within 7 days after disease onset in the index patient.

Limitations of the study

In this study we consider contacts with a positive IgM within 14 days after disease onset of the index case as co-primary cases. It is possible that some of the secondary cases with a longer incubation period were actually co-primarily infected. However, our study design is also used in other studies, so comparison with our results is not expected to be influenced by this assumption.

Conclusion

This study shows that IG does not protect all contacts from HAV infection. It does attenuate symptoms and reduces further spread of transmission: no tertiary cases were reported.

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Chapter 7

Molecular epidemiology of hepatitis A virus in Amsterdam, the Netherlands

Sylvia M. Bruisten (1); Jim E. van Steenbergen (2,3);
A.S. Pijl (1); Bert H.G.M. Niesters (4),
Gerard J.J. van Doornum (1,5), Roel A. Coutinho (2,6)

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1. GG&GD (Municipal Health Service), Public Health Laboratory, Amsterdam, The Netherlands (NL).
2. GG&GD, Dept. of Infectious Diseases, Amsterdam, NL.
3. LCI (National Co-ordination Communicable Disease Control), Utrecht, NL.
4. Erasmus Medical Centre, Dept. of Virology, Rotterdam, NL.
5. now at Erasmus Medical Centre, Dept. of Virology, Rotterdam, NL
6. Academic Medical Center, University of Amsterdam, Dept.of Human Retrovirology, Amsterdam, NL.

Abstract

Aim- To verify the transmission patterns of sporadic community-acquired hepatitis A virus (HAV) among different risk groups in Amsterdam by applying molecular techniques on fecal samples.

Methods- Samples were collected in 1997/1998 from 33 persons with HAV infection which was confirmed serologically. From 8 of these persons serial stool samples were collected. Performance of nested RT-PCR targeting the VP3-VP1 and VP1-P2a regions followed by sequence analysis established the duration of fecal HAV RNA excretion in stool and the epidemiological molecular relationships between patients.

Results- Samples of 31 patients were RT-PCR positive, of which 24 were positive for both regions. Fecal HAV shedding was found to occur for at least 33 days after onset of disease, which was the longest time span tested. Sequencing showed that the hepatitis A virus subgenotype circulating among persons from Moroccan descent (type IB) was different from the subgenotype circulating among Dutch homosexual men (type IA).

Conclusions- Molecular epidemiology might be a new powerful tool for public health. Further study has to confirm if indeed the subgenotype 1B is endemic in the Netherlands, as its presence is of importance to the national vaccination strategy.

Introduction

Hepatitis A virus (HAV) infection causes little or no disease in small children, but in adults it may lead to serious complications and even fatalities [1,2]. HAV is transmitted by the fecal-oral route, with person-to-person spread being common within families, schools, and other close-knit communities [1,3]. Due to public health improvements (clean water, sanitation) and better social economic conditions, the annual incidence of reported cases of hepatitis A has markedly declined in developed countries. In the Netherlands, where reporting is mandatory, the incidence declined from about 45/100.000 in 1952 to 6/100.000 at present [4]. The cases follow a seasonal pattern, with a peak in August and September. Epidemiological evidence suggests that children of immigrants who return from summer holidays spent in the parental country of origin, (often Morocco or Turkey), are the main importers of HAV in the Netherlands [5]. Secondary transmission within schools and daycare centers often occurs, although once an index patient is notified, advice on hygienic precautions and passive immunization of contacts is provided.
The development of a specific RT-PCR has increased the sensitivity of detection of HAV. It has been used to establish the duration of fecal excretion after the development of clinical symptoms in outbreaks of hepatitis A [6, 7, 8]. We performed a community-based study in Amsterdam in which sporadic cases of hepatitis A disease were recorded and patients were asked to participate. Multiple serial fecal samples, derived from 8 persons after the first manifestation of clinical symptoms, were analyzed to determine how long HAV RNA can be detected in feces using the RT-PCR. Single samples derived from 25 persons were also analyzed.
Using sequence analysis, the molecular subtypes of HAV that circulate in Amsterdam were determined in this study. Two regions were examined, one in the capsid proteins of HAV, VP3-VP1, and the other in the VP1-P2a junction, for which at least 7 unique HAV genotypes have been described worldwide [9, 10, 11]. In this study, these regions were used to determine the presence of possible endemic and imported hepatitis A virus in Amsterdam, the Netherlands. In addition, the usefulness of molecular epidemiology for contact tracing and identification of high incidence at risk groups was investigated.

Participants and methods

Participants
At the Municipal Health Service in Amsterdam, all reported cases of hepatitis A

are registered, and the patients are approached for active surveillance including source and contact tracing, and preventive intervention consisting of passive immunization of contacts and hygienic advice. Between January 1997 and March 1998 a total of 162 persons presented with hepatitis A disease. Of these, single fecal samples were collected from 25 persons with serologically confirmed, IgM anti-HAV positive, hepatitis A, including 4 persons from one family household. Samples were obtained on day 2 to day 33 after onset of disease as indicated by jaundice, or fever. In addition, serial daily stool samples were collected from 8 confirmed hepatitis A cases ranging from day 1 to day 29 after the appearance of clinical symptoms. Risk factors for acquiring HAV were defined as travelling to HAV-endemic countries, in particular to Morocco and Turkey, having school or household contacts with cases, and being men with homosexual contacts. The people who consented to participate in this study did not differ with respect to risk factors from those who declined to participate.

Isolation of RNA

Fresh feces samples were aliquotted as a 5%-20% suspension in a nutrient broth buffer (Oxoid Broth 2) containing 0.5% penicillin, 0.5% streptomycin and 0.3% fungizone and stored at –80°C until HAV RNA isolation was performed. The RNA was isolated by a method adapted from Chomczynski and Sacchi [12]. About 400 µl of feces suspension was thawed by adding 1200 µl lysis buffer containing 4.7 M guanidine thiocyanate, 46 mM Tris HCl pH 7.2, 1.2% Triton X-100 w/v, and 20 mM EDTA. After incubation at 65°C for 30 minutes, the samples were briefly centrifuged at 14000 rpm, (Eppendorf 5417C) at room temperature to remove insoluble debris. The supernatant was divided into two vials, one of which was spiked with 5 µl of HAV-positive culture supernatant. By spiking of a part of each stool sample with cultured virus (>10^6/sample) and processing it in parallel with non-spiked sample, RT-PCR inhibition was controlled for. This virus was a CPE inducing variant of strain HM175 (HAV cyt/HB1.1) kindly provided by dr. A. Dotzauer [13]. To each vial 1/10 volume of 3 M sodium acetate pH 5.0 and one volume of phenol/ chloroform/isoamylalcohol (25:24:1) was added. Vigorous shaking for 10 minutes at room temperature was followed by 2 minutes centrifugation (14000 rpm). The water phase was transferred to a clean vial and nucleic acids were precipitated with one volume of ice-cold 2-propanol. After centrifugation at room temperature for 20 minutes at 14000 rpm, (Eppendorf 5417C) the pellets were washed twice with 70% ethanol. The dried RNA pellet was dissolved in 500 µl of 10 mM Tris-HCl pH 8.0 and stored at –80°C until amplification was performed.

HAV RNA amplification

Reverse transcription was carried out in a volume of 25 µl using 5 µl of RNA solution, 1.2 ng of random hexamer primers, 2.5 units of MMLV RT enzyme (Gibco BRL), 1x first-strand buffer (50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$), 8 mM dithiothreitol, 10 units of RNasin (Roche Molecular Biochemicals), and 300 µM dNTPs (Roche MB). The cDNA reaction was stopped by adding 25 µl of distilled water. Two different nested PCR reactions were performed each in a volume of 25 µl. Each mixture contained 200 µM dNTP's, 2.0 mM $MgCl_2$, 1x PCR buffer, 0.5 units of SilverTaq Polymerase (Eurogentec, Seraing, Belgium) and 20 ng of each primer. To the first, outer PCR reaction, 5 µl of cDNA solution was added as template. To the inner primer mixture, 2 µl of outer PCR solution was added. Primers (Life Technologies, Breda, the Netherlands) were derived and modified from published sequences [9, 14] and are shown in Table 1.
Cycling conditions for both inner and outer reactions were: 3 minutes at 94°C, 30 cycles of 30 sec at 93°C, 30 sec at 55°C, 50 sec at 72°C and a final incubation of 7 minutes at 72°C.

Table 1

Primer sequences and location

Region	name	sequence 5'-3'	nucleotide number*	Fragment length
VP3-VP1	VP1-4 VP1-5	CGT.TGC.TTC.CCA.TGT.CAG.AG GAC.CTT.CCC.ATA.AAC.TTG.TAG	2115-2135 2483-2462	369 bp
	VP1-2^{+} VP1-1^{+}	GTT.TTG.CTC.CTC.TTT.ATC.ATG.CTA.TG GGA.AAT.GTC.TCA.GGT.ACT.TTC.TTT.G	2168-2194 2415-2390	247 bp
VP1-P2a	BR-5$^{\&}$ BR-9 $^{\&}$	TTG.TCT.GTC.ACA.GAA.CAA.TCA.G AGT.CAC.ACC.TCT.CCA.GGA.AAA.CTT	2950-2972 3310-3286	360 bp
	RJ-3$^{\#}$ BR-6$^{\&}$	TCC.CAG.AGC.TCC.ATT.GAA AGG.AGG.TGG.AAG.CAC.TTC.ATT.TGA	2984-3002 3217-3193	234 bp

*The numbering is according to Cohen et al [15].
Primer sequences VP1-1, VP1-2, BR-5, BR-9, RJ-3 and BR-6 were derived from published sequences, with a 5'-three nucleotides truncation in BR-5 [$^{+}$9; $^{\&}$10 $^{\#}$14]. VP1-4 and VP1-5 were chosen by the authors to develop a nested PCR.
Outer primer sets are VP1-4, VP1-5 for the VP3-VP1 region , and BR-5, BR-9 for the VP1-P2a region.
Inner primer sets are VP 1-2, VP 1-1 for the VP3-VP1 region , and RJ-3, BR-6 for the VP1-P2a region.
Each of the four inner primers was also used for sequencing analysis.

Sequencing analysis

To study the genetic relatedness between the HAV strains involved, a phylogenetic analysis was performed on two selected genomic regions of the virus, i.e., the C terminus of VP3 through the N terminus of VP1 (VP3-VP1) and the VP1-P2a junction. As a control isolate, HAV strain HM175 (HAV cyt/HB1.1) was used. The sequence analysis was carried out under code (i.e. blinded). Sequencing was performed directly on the nested PCR products, using inner primers and dye terminator chemistry on a Vistra Labstation (Pharmacia Amersham, Buckinghamshire, United Kingdom). Analysis was done on an Applied Biosystems 373 automated sequencer (PE Biosystems, Nieuwerkerk a/d IJssel, the Netherlands). Both strands were sequenced and analyzed using Geneworks software (Oxford Molecular, Oxford, United Kingdom). A neighbor-joining tree was constructed based on Kimura-2 parameter distances in the program MEGA. Published reference sequences were derived from GenBank and from Robertson et al. [10].

Results

Participants

The characteristics of the 33 participants are shown in Table 2. Most children belonged to families who originated from Morocco or Turkey. Since the incubation period of hepatitis A virus infection is two to six weeks, three patients (numbers 5, 10 and 13) most probably were infected abroad, because they had clinical symptoms of hepatitis A disease during their stay abroad or shortly after returning to the Netherlands. Patient 5 most probably acquired his infection in Turkey, whereas patients 10 and 13 acquired their infection in Morocco. Also, patients number 2, 7 and 9 may have acquired their infection directly in Morocco. All other children were infected in the Netherlands, at school or by household contacts (Table 2).

Six adult participants were men with homosexual contacts who visited sauna facilities or “dark rooms” in gay bars (patients 21-24, 26 and 28). Four other adults (patients 20, 25, 27 and 29) had various non-homosexual risk factors. Patient 20 is a 23-year-old female who visited South Africa, where she had lived previously. Patient 25 is a salesman of 33 years of age who had no clear risk factor for acquiring HAV. Although he became IgM-positive, he showed only mild disease symptoms and did not seroconvert to IgG positivity. His feces sample was repeatedly tested but persistently RT-PCR negative for both regions (Table 2). Patient 27 probably acquired his HAV infection from his adopted children who visited a school where hepatitis A disease was prevalent. Patient 29 most likely was infected in Algeria when she went there on a holiday to visit her family.

Table 2

Patient characteristics and results of HAV RT-PCR.

Pat Nr	sex	Age (yr)	country of birth	origin of parents	# feces	interval*	risk for acquiring HAV	HAV RT-PCCR VP3-1	HAV RT-PCCR VP1-P	Geno type
1	M	3	Netherlands	Morocco	1	4	school contact?	**pos**	**pos**	1B
2	F	3	Netherlands	Morocco	1	9	contact, Morocco	**pos**	**pos**	1B
3	F	6	Netherlands	Morocco	1	8	school contact?	**pos**	**pos**	*1B*
4	F	6	Netherlands	Morocco	1	7	contact, Morocco	**pos**	**pos**	*1B*
5	M	6	Turkey	Turkey	1	5	trip to Turkey	**pos**	**pos**	*1B*
6	F	8	Netherlands	Turkey	1	12	school contact	**pos**	**pos**	*1B*
7	F	8	Netherlands	Netherlands	1	5	contact, Morocco	**pos**	**pos**	*1B*
8	M	8	Netherlands	Morocco	1	2	school contact	**pos**	**pos**	*1B*
9	M	9	Netherlands	Morocco	1	33	contact, Morocco	**pos**	**pos**	*1B*
10	F	9	Netherlands	Morocco	1	5	trip to Morocco	**pos**	**pos**	*1B*
11	M	9	Netherlands	Turkey	11	1-11	patient 6 contact	**pos**	**pos**	*1B*
12	F	9	Unknown	Turkey	1	5	school contact	**pos**	**pos**	*1B*
13	M	10	Morocco	Morocco	1	12	trip to Morocco	**pos**	**pos**	*1B*
14	M	10	Netherlands	Morocco	7	6-16	school contact?	**pos**	**pos**	*1B*
15	F	10	Netherlands	Morocco	1	7	school contact?	neg	neg	*1B*
16	F	12	Unknown	Turkey	1	12	household contact	**pos**	**pos**	*1B*
17	M	13	Netherlands	Morocco	1	7	unknown	**pos**	**pos**	*1B*
18	M	16	Netherlands	Italy, Netherlands	1	6	heterosexual contacts	neg/neg	**pos**	**1A**
19	M	16	Netherlands	Turkey	1	6	school / household contact	**pos**	**pos**	*1B*

Pat Nr	sex	Age (yr)	country of birth	origin of parents	# feces	interval*	risk for acquiring HAV	HAV RT-PCCR VP3-1	VP1-P	Genotype
20	F	23	South Africa	NA	1	5	trip to South Africa	neg	**pos**	*1B*
21	M	29	Netherlands	Netherlands	8	6-24	homo/bisexual contacts	neg	**pos**	**1A**
22	M	30	Netherlands	Netherlands	9	14-27	homosexual partner is # 28	neg	**pos**	**1A**
23	M	32	Spain	NA	14	11-24	homosexual contacts	neg	**pos**	**1A**
24	M	33	Netherlands	Netherlands	14	7-20	homosexual contacts	neg	neg/**pc**	**1A**
25	M	33	Netherlands	Netherlands	1	9	work?	neg	neg	----
26	M	35	Netherlands	Netherlands	11	2-14	homosexual contacts	neg	**pos**	**1A**
27	M	35	Netherlands	Netherlands	1	10	household contact	**pos**	**pos**	*1B*
28	M	37	Netherlands	Netherlands	13	10-27	homosexual partner is # 22	neg	**pos**	**1A**
29	F	40	Netherlands	Netherlands	1	11	trip to Algeria	**pos**	**pos**	*1B*
30	F	4	Netherlands	Morocco	1	3	hepatitis A in family	**pos**	**pos**	*1B*
31	F	6	Netherlands	Morocco	1	27	hepatitis A in family	**pos**	**pos**	*1B*
32	M	10	Morocco	Morocco	1	21	hepatitis A in family	**pos**	**pos**	*1B*
33	M	12	Morocco	Morocco	1	7	hepatitis A in family	**pos**	**pos**	*1B*

Patients are sorted by age (in years). Patients 30 to 33 belong to the same family household.

NA: not available; # feces is the number of feces samples that has been analysed,

* is the time interval in days since onset of disease.

Pos is RT-PCR positive, neg is RT-PCR negative.

Duration of fecal HAV shedding

Of the 25 patients with single samples, 23 were HAV RNA-positive for both the VP3-VP1 region and the VP1-P2a region. The maximum duration of HAV RNA-positivity was 33 days after onset of disease (patient 9) which was the maximum time span tested in this study.

From the 8 persons with serial samples, the samples were taken on day 1 to day 27 after clinical symptoms appeared (Table 2). Of this follow-up group, 2 were children, a boy of Moroccan origin (patient 14) and a boy of Turkish origin (patient 11), whereas 6 were Dutch homosexual men. Patient 14 was RT-PCR positive for both primer regions up to the last sample, which was taken on day 16 after symptoms started (Figure 1). The family of patient 11 lived in the same house with the family of patient 6, a girl. The boy shed HAV RNA up to the last sample taken 11days after symptoms appeared; for the girl, only one sample taken at day 12 was available, but this was likewise HAV RNA-positive for both regions. The HAV sequences from these children were identical in both regions (see below). The follow-up samples derived from the 6 men with homosexual contacts were positive only for the VP1-P2a region. The samples of 2 of the 6 men were positive at all time points (patients 26 and 28, Figure 1). The maximum duration of fecal HAV shedding in these follow-up patients was 27 days (patient 28). Patient 28 was of interest because he suffered a clinical relapse with jaundice at 64 days after the first episode of hepatitis A disease. Again fecal samples were collected, ranging from day 5 to day 19 after recurrence of clinical symptoms. All of these samples were repeatedly HAV RT-PCR negative for both regions, indicating that the renewed jaundice was not associated with active HAV shedding.

If the single and follow up cases are taken together, the longest duration of HAV RT-PCR positivity was 33 days in this study, which was the maximum time span tested.

Figure 1

HAV-RT PCR amplification on follow-up samples of 8 persons, one child of Turkish and one of Morroccan origin and 6 Dutch homosexual men. Patient numbering corresponds to the numbering in Table II. Most persons had feces samples that were positive only for the VP1-P2a region (single positive: dashed blocks). Tested time points that were negative for both primer sets are indicated by blank blocks, whereas those that were double-positive, by black blocks.

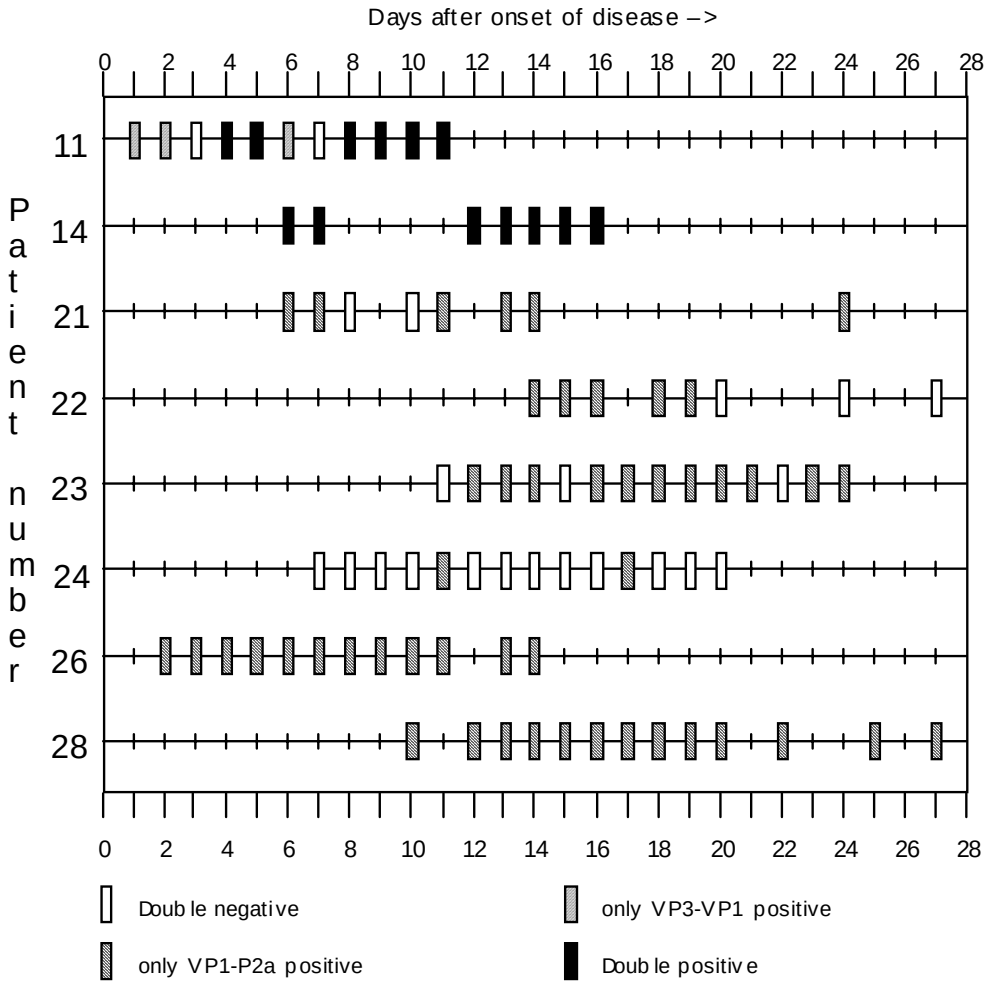

Figure 2

Neighbor-joining phylogenetic tree of the VP1-P2a region including sequences of patients of this study (in boldface), the reference virus HM175, and 9 reference sequences derived from the publication of Robertsen et al., 1992. Numbers indicate the reproducibility after 100 bootstraps, only bootstraps higher than 60 are shown. Genotypes are indicated. Bar length: 5% sequence diversity.

Sequencing analysis and molecular epidemiology

All positive RT-PCR products from patients with single samples and from follow-up patients with multiple samples taken at different time points were used for sequence analysis. Aligned sequences of 162 bp for the VP3-VP1 region and 160 bp for the VP1-P2a region were produced. Figure 2 shows the phylogenetic relationships for the VP1-P2a region, including 9 previously published sequences that are closely related to those of this study (genotype I) and one that is of an entirely different genotype (IIIA) [10]. The bootstrap values of the clusters vary from 60 to 87 per 100 replicates when the genotype IIIA strain is included, as is indicated in Figure 2. These values increase to 67-98% when the genotype IIIA strain is not included in the neighbor-joining bootstrap analysis.

The reference virus HM175 strain, subgenotype IB, which was used as an inhibition control in RT-PCR, differed from all other isolates that were sequenced by us. A subgroup of viruses that differed less than 1% in sequences from one another were of Moroccan origin, since they include the sequences of HAV isolates from patients (numbers 7, 9, and 13) who most likely acquired their infection while visiting Morocco. Patients 30 to 33 belong to one family household of Moroccan origin. The youngest patient (number 30) was the index case. Viral sequences of these children were 100% identical. Although patients 6, 11 and 12 had parents of Turkish origin, they all acquired their HAV infection in the Netherlands, most probably from Moroccan school mates, because their sequences clustered with those from patients that acquired their infection directly in Morocco. The isolate of Turkish patient 5 is closest related to that of patient 16, who is also of Turkish descent. Both cluster with an Algerian isolate, a South African isolate, and with reference strain HM175. All of our sequences differed less than 5% from other North African sequences that have been described as subgenotype IB [10].

With respect to risk factor, an exception among the children was patient number 18, a sixteen year-old boy who had not been abroad nor acquired his infection at school. He reported heterosexual contacts with many partners. His VP1-P2a sequence clustered with those of the 6 Dutch homosexual men (patients 21 to 24, 26, and 28) in whom these sequences differed less than 2% from USA sequences belonging to subgenotype IA (Figure 2).

From patients with follow-up samples, only one sequence was included in the dendrogram because in general, serial samples from each patient resulted in identical sequences for that patient. The first time point from patient 28 and the two time points from patient 24 were exceptions. The sample from patient 28 was not included because it may have been mislabeled. From patient 24, almost all serial samples were repeatedly RT-PCR negative, whereas all spiked samples were positive, indicating that the negative results were not due to sample inhibi-

tion. For only two samples (taken on day 11 and day 17) was there a positive RT-PCR. The sample from day 11 yielded a unique sequence, closely related to the sequences of the other homosexual men, whereas the sequence from day 17 was identical to that from patients 21, 26 and 28 (Figure 2).

For the VP3-VP1 region the phylogenetic pattern was similar to the pattern for VP1-P2a but there was no separate group of isolates for the Dutch homosexual men and patient 18 in this region, because the RT-PCR was negative for VP3-VP1 in all samples (Table 2). This probably indicates that one or more of the VP3-VP1 primers do not fit the HAV sequences present in the samples from the homosexual men or from patient 18. Again a group of Moroccan viruses, including those from patients 2, 7, 9, 10 and 13, could be assigned to subgenotype IB. The reference strain HM175 again ranked closest to the Turkish isolate of patient 5 for the VP3-VP1 region (not shown).

Discussion

Acute viral hepatitis used to be one of the commonest infections in children, seldom causing serious disease. Due to improvements in hygienic conditions its incidence has declined in the developed world, but the mean age of infection with HAV has increased, leading to an increased disease burden. The incubation period of hepatitis A is 15-50 days, with a mean of 30 days. Based on epidemiological studies, HAV is shed in feces for one to two weeks before the onset of illness and at least one week afterwards [1,3]. In the present study, persons with sporadic infections of hepatitis A who reported to the Municipal Health Service in Amsterdam were asked to participate by providing stool samples. Most cases were children of parents from ethnic minority groups, but some were adults with a Dutch background. In the present study HAV RNA shedding could be detected for many participants by RT-PCR in feces for at least one month after onset of disease. In a previous study, the duration of HAV positivity was reported to last up to 3 months in adults, although only the fecal samples collected within 10 days after symptoms were invariably positive, and only 5 of 10 persons tested were positive at all [8]. In another study, infants from a neonatal intensive care unit were found to excrete HAV RNA in feces for as long as 4-5 months after the infection [6]. This finding was based on two preterm infants, of whom one was followed for 7 months after infection. Possibly the lack of a fully functional immune system in these infants contributed to the prolonged excretion. On the whole, the number of participants and the proportion of HAV RNA-positive persons in our study was larger than previously reported. The longest time period

assessed by us was 33 days. This concerned a sample from an immuno-competent child whose fecal sample was positive for both viral regions tested. Possibly the duration of HAV RNA positivity can indeed last several months, but to what extent excretion of viral RNA makes an individual infectious to others is unclear. Since Tamarin monkeys can be infected with HAV by exposure to feces derived from HAV-infected persons up to 34 days after start of IgM positivity, the presence of viral sequences could indicate viral infectivity [16].

Fecal samples from three persons in our study group were repeatedly negative for HAV by RT-PCR. Two of these persons donated only one sample shortly after onset of disease, the other person (patient 24) had 14 serial samples ranging from day 7 to 20 after onset of symptoms. Of these serial samples, the RT-PCR was negative with both primer sets, with the exception of two samples that were positive with only the VP1-P2a primers. Sequence analysis showed that these two isolates differed. One isolate was identical to the isolates of the homosexual men in our study whereas the other was unique, but still related to the sequences from the homosexual men. One explanation for the negative findings in the 3 patients is that the viral load was very low in the feces samples of these persons. Alternatively, the primers did not recognize these viruses, or these persons were actually not HAV-infected at all. Which of these explanations is most likely cannot be decided from the available information. One of the 2 persons from whom only one sample was tested, had a positive IgM anti-HAV test but no clear clinical symptoms nor any obvious risk factors for hepatitis A, and he did not seroconvert for HAV IgG. Possibly the positive IgM anti-HAV test was due to an aspecific reaction connected with a reactivation of latent EBV infection [17]. The other was a 10-year-old Moroccan girl, born in the Netherlands. Her risk factor for acquiring HAV infection was a school contact with a hepatitis A-positive person.

Sequencing of variable regions within the capsid proteins of wild-type HAV isolates from different regions of the world revealed nucleic acid heterogeneity with limited amino acid heterogeneity [14, 9, 10, 11, 18]. On the basis of the nucleic acid heterogeneity, HAV isolates could be differentiated into 7 genotypes. A genotype is defined as a group of viruses that differ at no more than 15% of their base positions, and a subgenotype as having less than 7.5% heterogeneity [10]. In Western Europe, HAV isolates have been identified from multiple and possibly imported genotypes [10]. Such genetic analyses can provide valuable information with regard to the source of the virus in both sporadic and epidemic infection [11, 19, 20]. In the present study, sequence analysis was performed under code (i.e., blinded to the sequencing laboratory) and found to be highly reproducible: all time points tested in 6 follow-up cases yielded identical sequences in each case.

There was a clear distinction between the viruses derived from the group of homosexual men and the sequences derived from the immigrant children who were mostly from Morocco. The reference virus (HM175) belonged to subgenotype IB and clustered closest to the Turkish/Moroccan viruses. The virus sequences derived from the Dutch homosexual men clustered most closely with isolates from the USA belonging to subgenotype IA [10]. Previous studies by our group [21] and others [22] have found homosexual men to be a high risk group for hepatitis A virus infection, but in these studies no molecular epidemiology was performed.

The reservoir of the hepatitis A virus that circulates among the Dutch homosexual men is not known. Its low incidence among them and other people with a Dutch background suggests an ongoing unnoticed transmission by a series of asymptomatic infections. Ano-oral contacts, especially those performed sexually in “darkrooms” in gay bars by homosexual men are epidemiologically associated with HAV transmission [23]. The extent to which subgenotype IA virus is present also in drug abusers and other adults in the Netherlands requires further study. Scandinavian studies on HAV infection among drug users have shown identical sequences of the outbreak strains but divergence of up to 10 % between these and the strains isolated from non-drug users [24, 25]. It is also interesting to see whether the virus is sporadically introduced in the Dutch population by food or by contact with sewage [26, 27].

Our molecular typing results confirm that import of HAV into the Netherlands occurs, but also endemic strains could circulate. High incidence at risk groups can benefit from this knowledge by a targeted vaccination strategy, which is presently practiced in the Netherlands. Future studies of transmission routes among risk groups will provide a basis on which to decide whether universal or targeted vaccination is the best option for the Netherlands. Studies such as these also show that molecular epidemiology is a powerful tool to trace viral transmission through the community and provide evidence for contact tracing.

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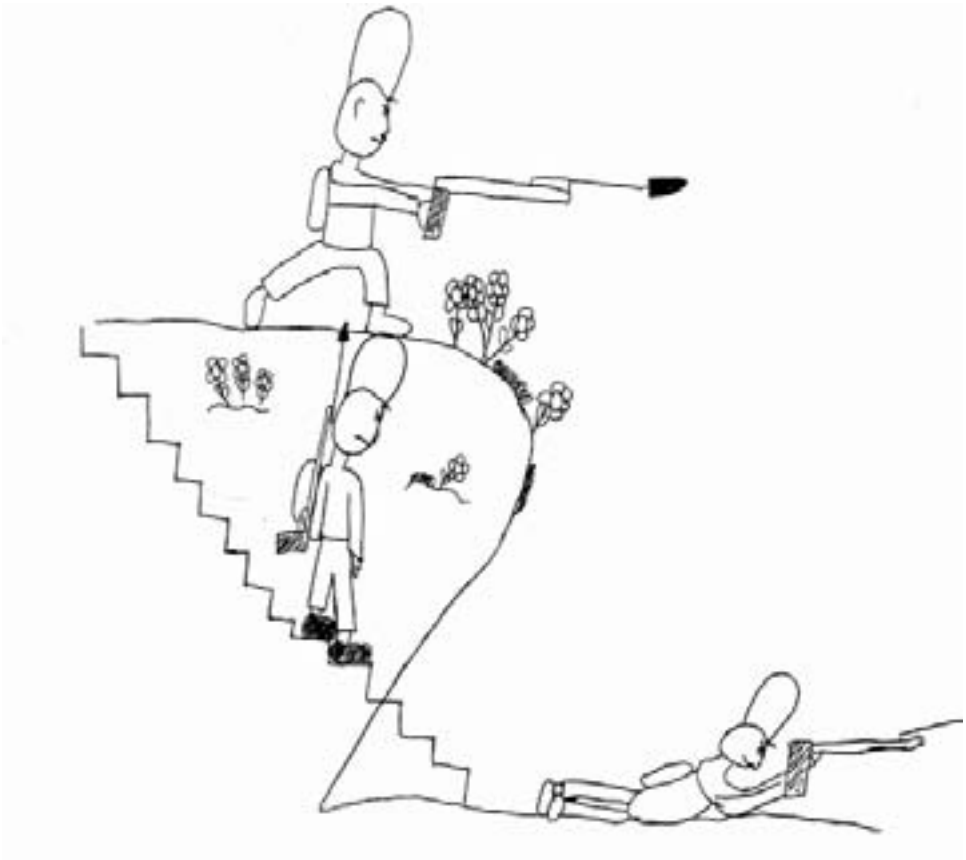

The importance of acute hepatitis to military forces is reflected in some of the names which have been employed to describe this illness: Jaunisse des camps, Kriegsickterus, Soldatengelbsucht, Campaign jaundice.
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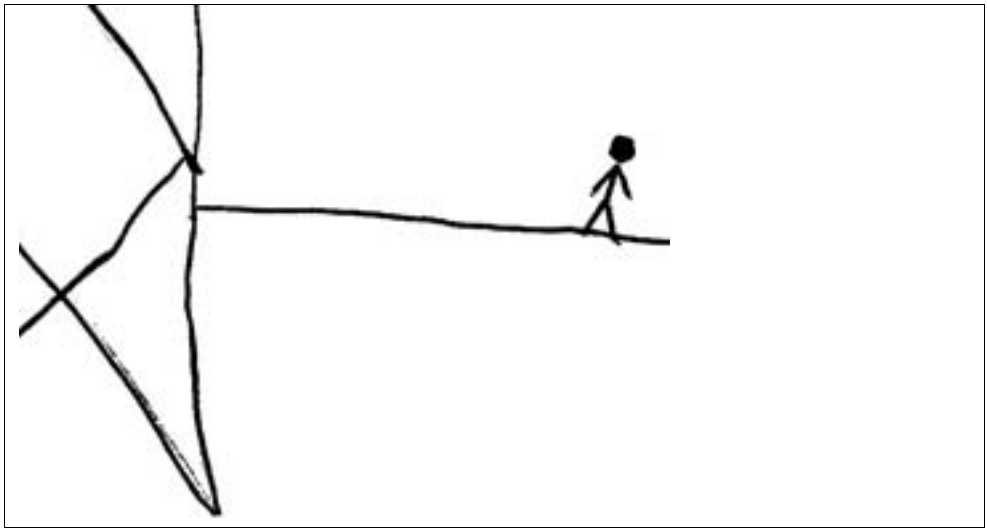
Chapter 8

Two years' prospective collection of molecular and epidemiological data shows limited spread of hepatitis A outside risk groups in Amsterdam 2000-2002

Jim E. van Steenbergen (1,3), Grace Tjon (2), Anneke van den Hoek (1), Alex Koek (2), Roel A. Coutinho (1,4), Sylvia M. Bruisten (2)



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1. GG&GD (Municipal Health Service), Dept. of Infectious Diseases, Amsterdam, The Netherlands (NL)
2. GG&GD, Public Health Laboratory, Amsterdam, NL.
3. LCI (National Co-ordination Communicable Disease Control), Utrecht, NL
4. Academic Medical Center, University of Amsterdam, Dept. of Human Retrovirology, Amsterdam, NL.

Abstract

Aim- To gain insight in the transmission routes of hepatitis A in Amsterdam to enable policy decisions regarding vaccination policy.

Methods- A viral sequencing study was performed on samples representing all reported primary cases of acute hepatitis A virus (HAV) infection reported over two years in Amsterdam. Two regions of HAV RNA were amplified and sequenced and used for phylogenetic analysis.

Results- Of 156 cases, strains of 104 isolates (66%) clustered into three genotypes: 1A, 1B and 3. Two separate transmission circles occurred, without mutual interrelation. In genotype 1A, four clusters were related to men having sex with men, the fifth related to a virus imported from Morocco. In genotype 1B, six small clusters were directly related to the Moroccan import. In genotype 3, import cases were related to Pakistan.

Conclusions- Our analysis indicates that, to stop HAV transmission in Amsterdam, the entire homosexual population should be vaccinated, as well as travelers to HAV-endemic countries, especially children. Prevention strategies need not to include vaccination of all children living in Amsterdam.

Introduction

In most of the world hepatitis A virus (HAV) infection is known as an innocent, often asymptomatic childhood disease. In the rich industrialized world, however, with decreased crowding and increased hygiene and public health interventions, HAV infection occurs at a later age, with increased morbidity and mortality over the age of 40 years [1,2]. In the Netherlands, where symptomatic HAV cases are notifiable, the incidence of reported cases has varied in the last decade from 4.1 to 7.9/100.000 [3]. The incidence of reported cases in Amsterdam was 23.1/100.000 from 1991-2000, with lower incidence in the last four years [4,5]. Amsterdam is assumed to have approximately 735.000 inhabitants, of whom 37% originate from developing countries where HAV is endemic. Enhanced surveillance, in the four largest cities of the country, suggests that children of this subpopulation import HAV on return from travel to the country of origin, causing secondary transmission within their family, schools or daycare centers [6]. Susceptible household members of notified cases are immunized with immune globulin. If a cluster of cases points to transmission in a school or daycare center, immune globulin is administered to susceptible group- or classmates and sometimes to their relatives. We started annual hepatitis A vaccination programs in 1998, for children visiting their country of parental origin, but achieved vaccination coverage of less than 50% for children under 16 years of age [7]. Also, school-related clusters continued to occur [4,5]. Since vaccine costs seem to argue against universal childhood vaccination in the Netherlands [8], we sought a targeted approach, by investigating the molecular epidemiology of HAV in Amsterdam. We isolated and sequenced HAV RNA from stool samples in a pilot study of 33 acute index cases in 1997 and 1998, finding two distinct subtypes: genotype 1B introduced by children from Morocco and genotype 1A endemic among homosexual men [9]. Now, based on all incident reported cases in a two-year period, we present a complete picture of the molecular epidemiology of HAV in Amsterdam. Our findings are important for policy decisions regarding the most effective HAV prevention strategy.

Participants and methods

Participants

From August 1, 2000 to August 30, 2002, 156 index patients with community-acquired acute symptomatic HAV infection were notified to the Municipal Health Service (MHS), Amsterdam. An additional 26 individuals, with serologically confirmed acute infection, were traced as contacts. Reporting criteria are clinical

signs and symptoms with laboratory confirmation of acute infection as measured by the presence of type M immune globulin antibodies to HAV (antiHAV-IgM). Reported patients are typically approached with active surveillance including source- and contact-tracing, passive immunization of susceptible contacts at risk, and hygienic advice. Information is collected on possible risk factors during the two to six weeks preceding disease onset. For our study individuals were classified with an hierarchical algorithm as to probable mode of transmission. The first group are individuals returning from HAV-endemic countries (IMP), two to six weeks prior to disease onset. Second are people with a confirmed hepatitis A patient in the household or family (FAM). Third are children having a school contact with a confirmed case (SCH). Fourth are men having sex with men (MSM) visiting “darkrooms” or other venues for anonymous sex. Fifth are drug users, of whom drug type, frequency and route of administration is registered. The sixth group includes patients for whom none of these risks are identified and transmission is classified as "unknown" (UNK). Besides transmission risk factors, we recorded data for each case including age, gender, country of origin, food history (especially regarding fresh berries, shellfish, and possibly contaminated water sources) and the identified source person if any. With informed consent of reported patients and 26 contacts, we tried to retrieve serum and/or plasma samples from the diagnosing laboratory. Subjects were asked to send stool samples to our laboratory.

Isolation, amplification, and sequencing

RNA Isolation

Serum and plasma samples were aliquoted in 1.7 ml vials, and stool samples were aliquoted as a 10 to 20% suspension in phosphate buffered saline. All samples were stored at minus 80°C until isolation of RNA was performed, using TriPure Isolation Reagent (Roche), according to the manufacturer’s protocol. Isolated RNA was resuspended in 50 µl Tris HCl (10mM, pH 8.0) and stored at -80°C. As a control for the RNA isolation and the subsequent nested reverse transcriptase polymerase chain reaction (RT-PCR), one sample was spiked with 5 µl of a HAV-positive culture supernatant (HAV cyt HB1.1 ; [10]) and processed in parallel with the studied serum, plasma and stool samples.

Oligonucleotide primers

Random hexamer primers were used for reverse transcription (RT). PCR primers targeting the VP1-P2a region and the more variable VP3-VP1 region of the HAV genome were derived and modified from published sequences (Table 1) [9,11-

13]. The second-round PCR was performed with primers located internal to the first round PCR primers. All primers were synthesized by Life Technologies (Gibco BRL, the Netherlands).

Table 1

Primer sequences and locations in the HAV genomic regions VP1-P2a and VP3-VP1

Region	Primer	Sequence 5'-3'	Nucl. nr.[14]	Size (bp)
VP1-P2a	1st round PCR			
	BR-5[13] sense	TTG TCT GTC ACW GAA CAR TCW G*	2950-2972	360
	BR-9[13] antisense	AGT CAC WCC TCT CCA RGA AAA YTT*	3310-3286	
	2nd round PCR			
	RJ-3[11] sense	TCC YAG AGC WCC WTT RAA*	2984-3002	218
	BR-7 antisense	ACT TCA TTT GAC AAC TCT TCC TGA	3179-3202	
VP3-VP1	1st round PCR			
	VP1-4[9] sense	YGT TGC TTC YCA TGT YAG AGT*	2115-2135	341
	VP1-6 antisense	CAT ATG ATC TGA TGT ATG TCT	2436-2456	
	2nd round PCR			
	VP1-2[12] sense	GTT TTG CTC CTC TTT ATC ATG CTA TG	2168-2194	247
	VP1-1[12]antisense	GGA AAT GTC TCA GGT ACT TTC TTT G	2390-2415	

*R=A+G; W=A+T; Y=C+T

Coded primer sequences were derived and modified from published articles as indicated in the list of references. The nested PCR primers were used also for sequencing analysis.

HAV RNA amplification

Nested RT-PCR was performed in a PTC-200 DNA Engine Thermal Cycler (MJ Research Inc., through BiozymTC BV, Landgraaf, the Netherlands) as described previously [9]. Sequencing was performed directly on the second round PCR products using the second-round primers and Big Dye Terminator chemistry v2.0 and v3.0 (PE Biosystems, Nieuwerkerk a/d IJssel, the Netherlands).

Sequencing and phylogenetic analysis

Sequencing products were analyzed on an ABI 310 automated sequencer (PE Biosystems, Nieuwerkerk a/d IJssel, the Netherlands) and aligned with the BioEdit Sequence alignment Editor computer program [15]. Aligned sequences were then used to generate a phylogenetic tree using Molecular Evolutionary Genetics Analysis (MEGA) computer software version 2.1 [16]. The tree was constructed using neighbor joining and Kimura-2 parameter models, and repro-

ducibility was tested performing 1000 bootstraps.

Nucleotide sequence accession numbers
The nucleotide sequence data reported in this paper have been deposited in the GenBank sequence database under accession no. AY343685 to AY343785, AY101267 to AY101271, AY343786 to AY343888, and AY101276 to AY101280. The reference types used were obtained from GenBank (www.ncbi.nlm.nih.gov) genotype 1A (Accession no.: X75214; AB020564), genotype 1B (Accession no.: M59808; M20273), genotype 3A: (Accession no.: M66695).

Statistical analyses
Where appropriate, Chi-square test was used, to compare characteristics between groups. To calculate risk factors for non-availability of HAV-RNA sequences, SPSS logistic regression was used to obtain univariate and multivariate odd's ratio's (ORs), and 95% confidence intervals (CIs). In multivariate modeling, all relevant factors were included.

Results

Participants
Of 156 reported hepatitis A index cases 120 were male (77%), and 73 were of non-Dutch origin (47%). Index cases had a mean age of 24,6 years (range 1-69 years). Of these, 61 were MSM having a mean age of 38.0 years (range 20-63 years). No drug use was reported. Five individuals were hospitalized (one to ten days). The percentage of jaundice was lower in children under the age of ten years (37/44, 84%) and higher in adults over 40 years of age (27/28, 96%). Of 110/156 index patients and 14/26 contact cases, at least one sample type was obtained (serum, plasma or feces, or a combination of samples). HAV RNA was sequenced from 14 contact samples, but these were not included in the analysis. In 10 contact cases, HAV RNA was sequenced both in index and contacts. Nine sequence pairs showed homology, but in the tenth, we identified a minor difference in the VP1-P2a region. Of four contacts, the index was not participating. Based on the homology in nine of ten index/contact pairs, we assumed the HAV RNA of these four contacts without index, to be identical to the non-participating index and added the samples into the index group. We thus analyzed 114/156 samples (73%). Index cases with available sequence represented in the phylogenetic tree in Figure 1a, were 78% male, 47% of non Dutch origin. Table 2 lists the age specifications per risk group of these participants.

Table 2

Availability of sequences of reported cases with acute hepatitis A in Amsterdam, according to source of transmission, with respective average age and age distribution of the separate transmission groups.

Probable Source of infection	Number index cases	Available sequences in VP1-P2a region Number/ (% available)	Age (average)	Age (range)	(standard deviation)
Import	48	29 (60)	12	1-38	11,7
Family	9	8 (89)	24	7-42	17,3
School	9	8 (89)	8	6-10	1,4
MSM activity	61	42 (69)	40	27-63	8,4
Unknown	29	17 (52)	22	4-36	12,8
Total number	156	104 (67)	25	1-63	17

Isolation and sequencing analysis

In 104/114 samples (91%) HAV RNA could be isolated and sequenced for the more conserved VP1-P2a region. The percentage was 94 (65/69) for individuals with two sample types, and 84 (45/52) for individuals with one sample type. For the more variable VP3-VP1 region, sequence could not be determined for 8 individuals (positivity rate 93%). Table 2 shows the probable transmission source for 156 reported cases related to the availability of a sequence in the VP1-P2a region. Availability was not biased towards gender (p>0,4) country of origin (p>0,8), source of transmission (p >0,25), or study period (p>0,5). A sequence was available in a lower percentage of adults 20 to 29 years (4/14, 29%), but this was not statistically significant (p=0,05). In a logistic regression model, including all above variables, availability of sequences was statistically not related to any of these variables. For both regions HAV RNA sequencing results were identical when derived from different samples (feces or blood) from the same individual.

Molecular epidemiology

HAV sequences from the VP1-P2a region are displayed in a phylogenetic tree constructed by the neighbor-joining method (Figure 1a), which includes reference sequences for the distinct genotypes. Overall, strains clustered in three genotypes: 1A, 1B, and 3. The phylogenetic tree based on sequences of the VP3-VP1 region is shown in Figure 1b. The sequences from the less conserved VP3-VP1 region showed a pattern of clustering similar to that of the VP1-P2a region, but, as expected, with more variability. Figures 2a, 2b, 2c respectively show subsets

of sequences in genotype 1A, 1B and genotype 3 of the VP1-P2a tree. Each sequence is designated by calendar year and week of disease onset, followed by source of transmission (imported IMP; family/household FAM; school SCH; visiting darkrooms MSM; unknown UNK).

Genotype 1A

Genotype 1A contains a cluster (MSM1 at bottom of Fig 2a) consisting of 10 isolates from Amsterdam. Detected from the start of sample collection, in week 31 in 2000, through week 12 in 2001, all were derived from MSM: 9 with high-risk homosexual contacts and one classified import (IMP), as he returned from a one month travel to Australia, with disease onset the day before return.
The cluster SCH1 (Fig 2a), consists of a case in a Moroccan child reported with onset in week 37 in 2000. A similar strain was isolated in an epidemiological cluster that occurred 15 months later, consisting of several distinct household contacts (FAM) related to a primary school.
The largest cluster in genotype 1A, (MSM2) consists of 33 isolates, and covers 53 weeks, January 2001 through January 2002. In 31/33 cases the patient reported homosexual contact, of whom 28 high-risk behavior in darkrooms. Three homosexual men were differently classified (“~” in Fig 2a): one import (IMP), who traveled 10 days in the Republic of South Africa, three weeks preceding his illness; one partner (FAM) of a HAV patient, living outside Amsterdam (not reported); one unknown (UNK), as he reported no high-risk behavior, but only kissing with men. Of the two non-MSM in this cluster (“#” in Fig 2a), one traveled with his family to Spain, was classified UNK as he reported no specific risks for hepatitis A; the second was UNK, although transmission from a homosexual HAV patient at the workplace was speculated (patient living outside of Amsterdam, not included in this study). Isolated IMP strains in genotype 1A, were virus from various parts of the world (as indicated in Figures 2).

Genotype 1B

In Figure 2b, the sequences of genotype 1B show more variation, four clusters consisting of two or three identical strains and two with five and six identical strains. Cluster SCH3 started with IMP from Morocco, and could be followed in a primary and secondary school. Two cases were classified UNK, as transmission at secondary school was only speculated. Three cases were non-traveling children of Dutch origin, later followed by contact cases among their parents (contact data not shown). A sixth virus, on epidemiological grounds related to this cluster, shows a sequence with minimal nucleotide differences (2001WK50_SCH). A cluster of two cases (marked “+” at bottom Fig 2b) consisted of a child (IMP), and his nephew (FAM) with low-risk contact. In an epidemiologically defined cluster,

related to a day care center, where the MHS intervened with immune globulin, no relation with import of virus was ascertained.

Genotype 3

While most imported cases in genotype 1 originated from Morocco, those of genotype 3 (Fig 2c) originated from Pakistan. One family contracted a genotype 3 strain, probably in Spain. The index case was classified IMP (2000WK40_IMP). In genotype 3, two isolated cases of individuals with unknown source were found. One was a woman, whose only possible risk was taking care of a baby of Ethiopian origin, without any symptomatic hepatitis A cases. The second was a man, living on the first floor of an apartment building, who repaired the leaking sewage pipe, running through his shed, six weeks prior to his illness. From the same apartment building, a hepatitis patient was reported, living on the third floor. He was a flight steward, who, due to his frequent absence from home, was not traced in time to consent retrieval of serum samples from the diagnosing laboratory.

Figure 1a

Neighbor-joining phylogenetic tree of the nucleotide sequences in the VP1-P2a region of isolates of 104 acute hepatitis A cases from individuals in Amsterdam. The tree includes reference viruses GBM and AH-1 in genotype 1A, HM175 in genotype 1B, and GA76 in genotype 3A. Sample numbers show successive entry number and origin. The two-digit numbers in the figure represent the bootstrap values obtained by generating 1000 trees. Only bootstrap values of 70 and higher are shown. The samples are numbered in order of arrival in the laboratory.

MSM1, a cluster of 10 isolates with 100% similarity
MSM2, a cluster of 33 isolates with 100% similarity
SCH1, a cluster related to a primary school of six isolates with 100% similarity
SCH2, a cluster related to a primary school, of four identical strains
SCH3, a cluster related to a primary school, of five identical strains

Figure 1a

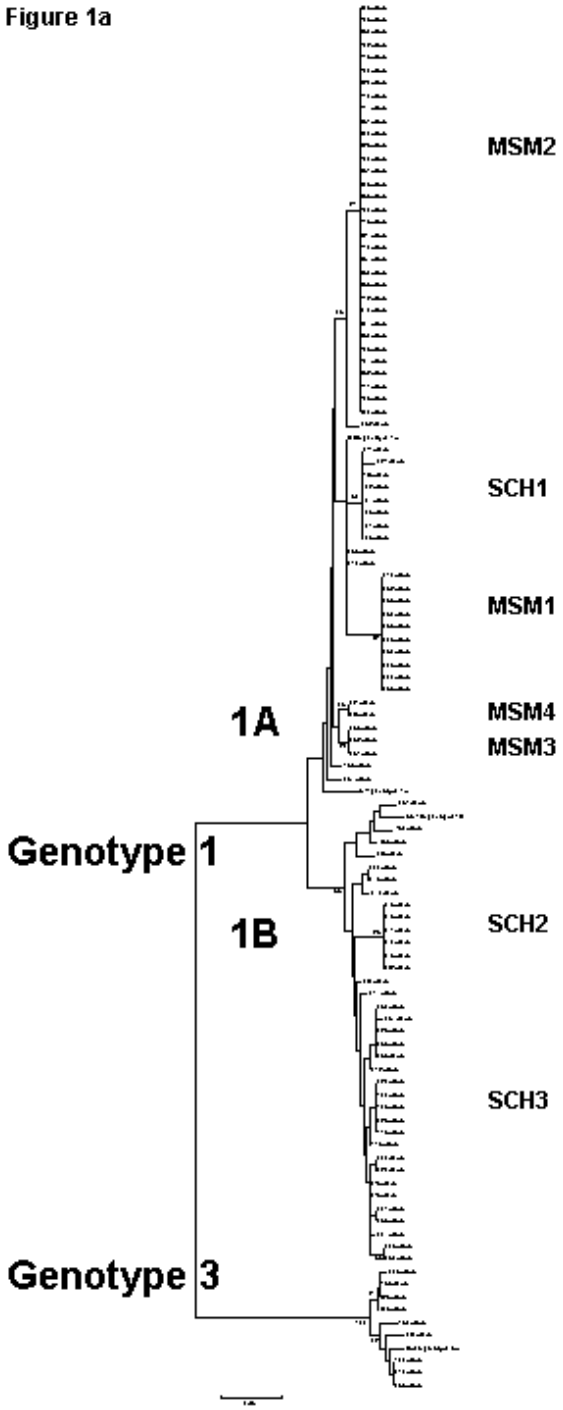

VP1-P2a region
MSM2
SCH1
MSM1
MSM4
MSM3
1A
Genotype 1
1B
SCH2
SCH3
Genotype 3

Figure 1b

Neighbor-joining phylogenetic tree of the nucleotide sequences in the VP3-VP1 region of the same isolates as in figure 1a, including the same reference viruses, with similar representation of bootstrap values.

Figure 1b

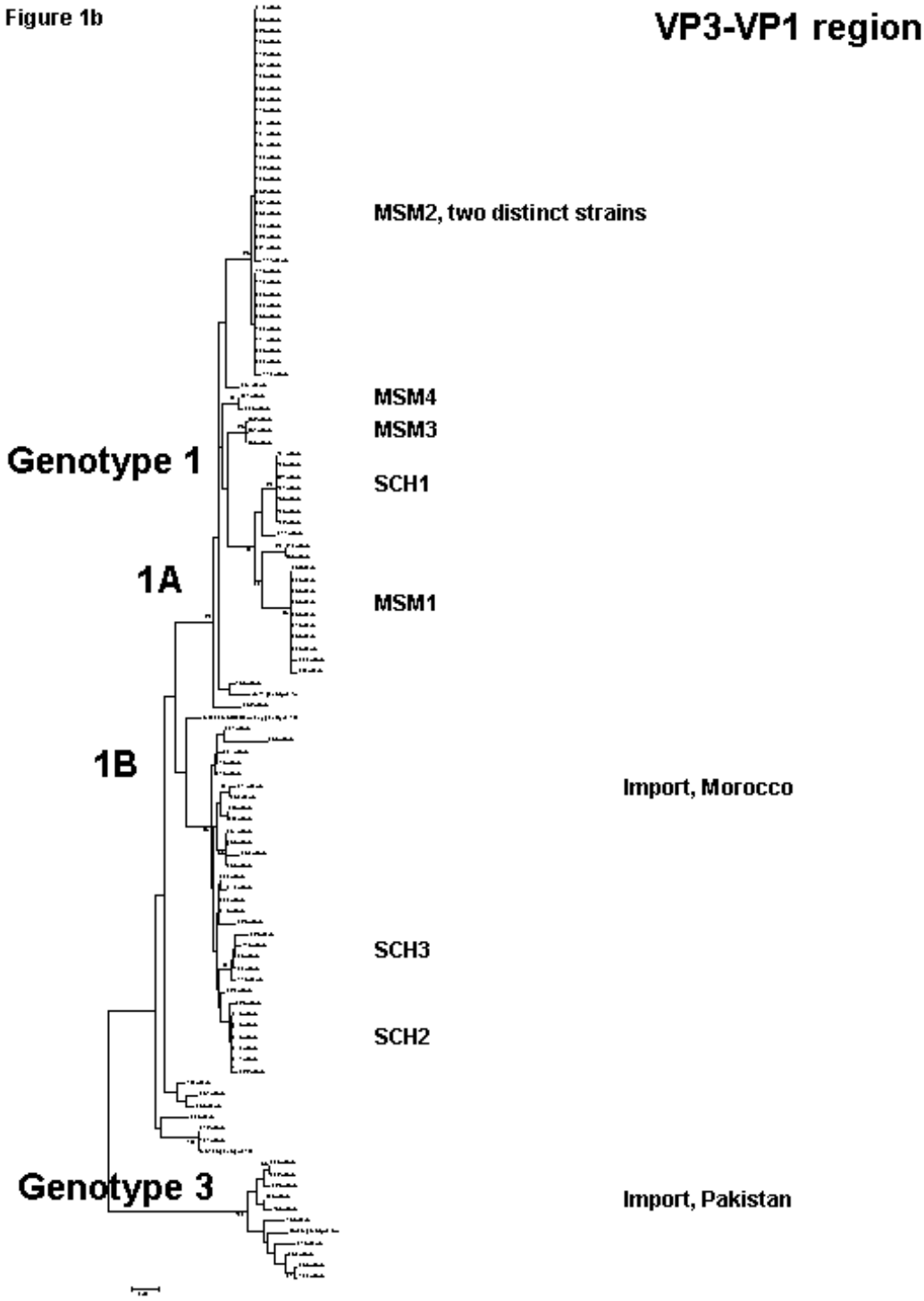

VP3-VP1 region
MSM2, two distinct strains
MSM4
MSM3
Genotype 1
SCH1
1A
MSM1
1B
Import, Morocco
SCH3
SCH2
Genotype 3
Import, Pakistan

Figure 2a

Subset of genotype 1A from the neighbor-joining phylogenetic tree of the nucleotide sequences in the VP1-P2a region of figure 1a. The sample numbers indicate the year and week of disease onset and probable source of transmission

IMP : returning from areas of high/medium HAV endemicity in the six weeks preceding disease onset

FAM : family or household contact of hepatitis A case

SCH : school-related, i.e., acute hepatitis A in children from the same classroom or using the same toilet facilities in school

MSM : high-risk homosexual behavior in the six weeks preceding illness (predominantly high-risk sexual techniques during visits in darkrooms)

UNK : unknown source of transmission

In clusters of 100% similarity, isolates are represented in chronological order.

~ : Isolates from MSM not engaging in high-risk behavior

: isolates from non-MSM, with strains in MSM clusters.

Figure 2a

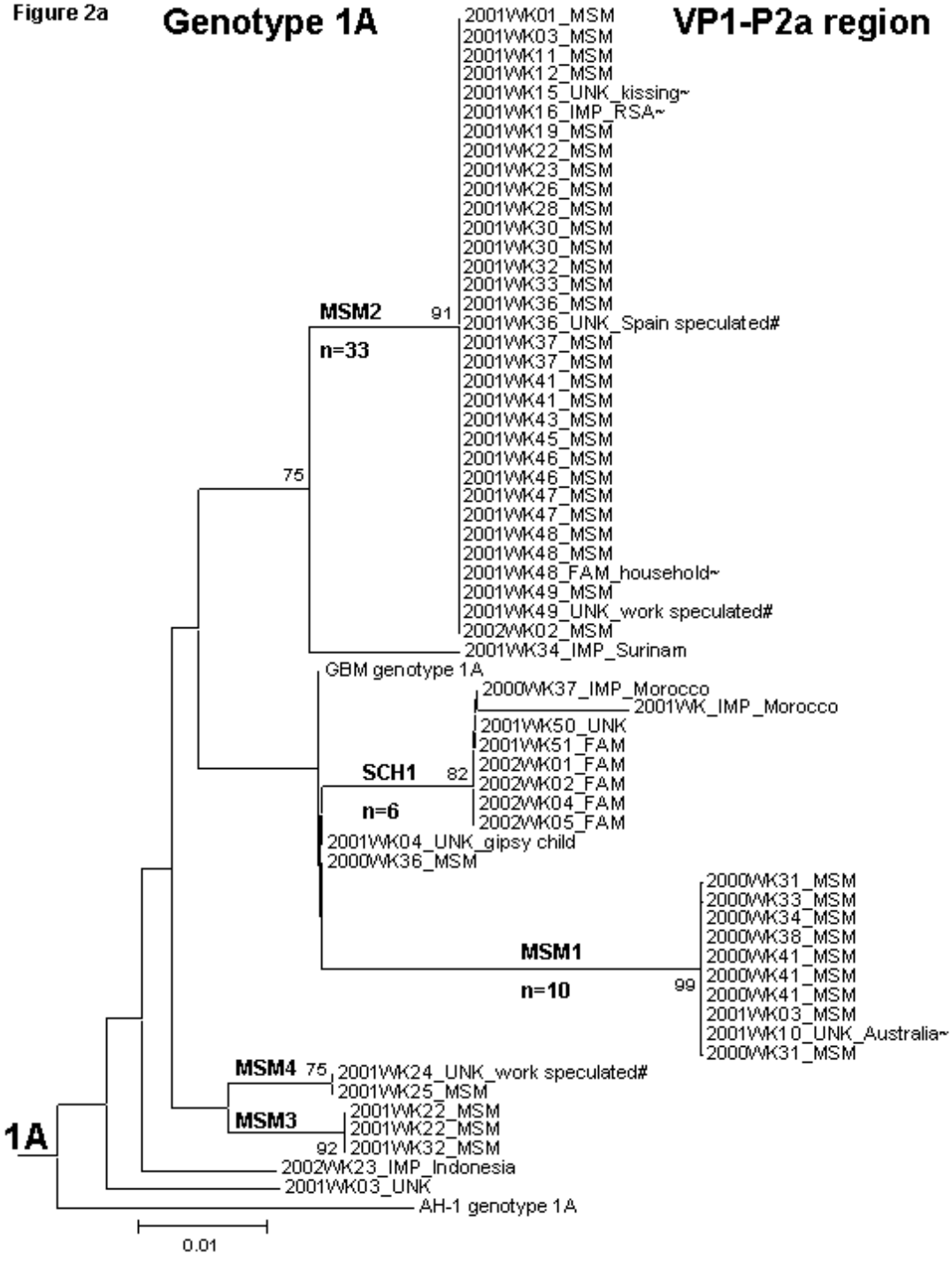

Genotype 1A
VP1-P2a region
2001WK01_MSM
2001WK03_MSM
2001WK11_MSM
2001WK12_MSM
2001WK15_UNK_kissing~
2001WK16_IMP_RSA~
2001WK19_MSM
2001WK22_MSM
2001WK23_MSM
2001WK26_MSM
2001WK28_MSM
2001WK30_MSM
2001WK30_MSM
2001WK32_MSM
2001WK33_MSM
2001WK36_MSM
MSM2
91
2001WK36_UNK_Spain speculated#
n=33
2001WK37_MSM
2001WK37_MSM
2001WK41_MSM
2001WK41_MSM
2001WK43_MSM
2001WK45_MSM
2001WK46_MSM
75
2001WK46_MSM
2001WK47_MSM
2001WK47_MSM
2001WK48_MSM
2001WK48_MSM
2001WK48_FAM_household~
2001WK49_MSM
2001WK49_UNK_work speculated#
2002WK02_MSM
2001WK34_IMP_Surinam
GBM genotype 1A
2000WK37_IMP_Morocco
2001WK_IMP_Morocco
2001WK50_UNK
2001WK51_FAM
SCH1
82
2002WK01_FAM
2002WK02_FAM
n=6
2002WK04_FAM
2002WK05_FAM
2001WK04_UNK_gipsy child
2000WK36_MSM
2000WK31_MSM
2000WK33_MSM
2000WK34_MSM
2000WK38_MSM
2000WK41_MSM
MSM1
2000WK41_MSM
n=10
99
2000WK41_MSM
2001WK03_MSM
2001WK10_UNK_Australia~
2000WK31_MSM
MSM4
75
2001WK24_UNK_work speculated#
2001WK25_MSM
MSM3
2001WK22_MSM
2001WK22_MSM
92
2001WK32_MSM
1A
2002WK23_IMP_Indonesia
2001WK03_UNK
AH-1 genotype 1A
0.01

Figure 2b

Subset of genotype 1B from the neighbor-joining phylogenetic tree of the nucleotide sequences in the VP1-P2a region of figure 1a. The sample numbers indicate the year and week of disease onset and probable source of transmission (as in 2a).

* : isolates of contact cases with strains that did not have 100% similarity

\+ : isolates with possible epidemiological relation, confirmed by molecular virology of isolates.

Figure 2b

Genotype1B, VP1-P2a region

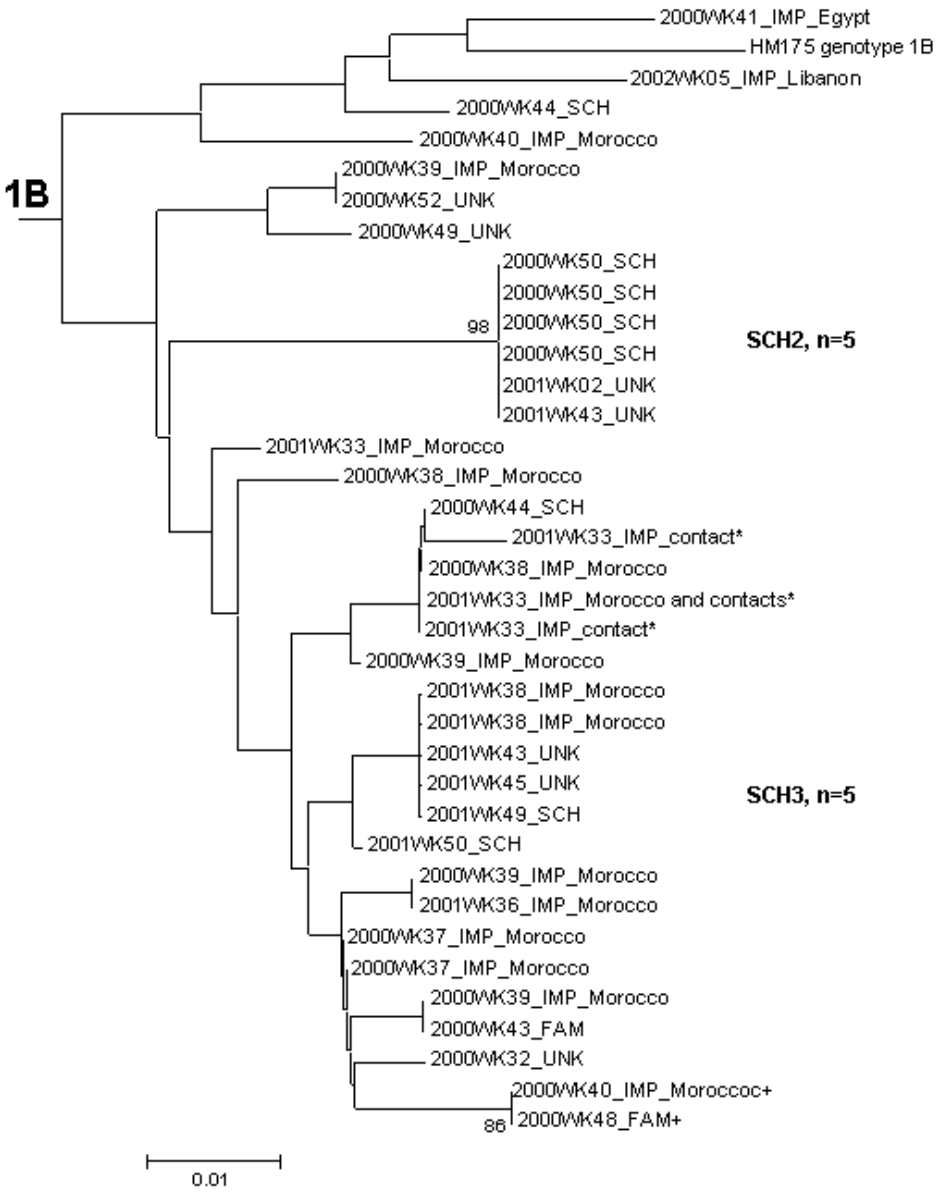
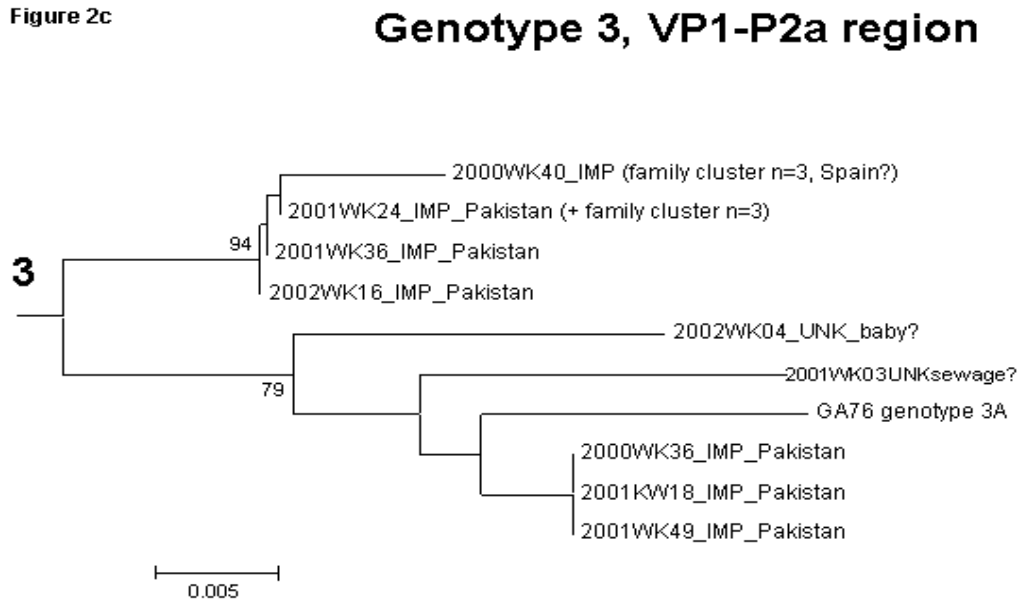


Figure 2c

Subset of genotype 3 from the neighbor-joining phylogenetic tree of the nucleotide sequences in the VP1-P2a region of figure 1a. The sample numbers indicate the year and week of disease onset and probable source of transmission (as in 2a).



Discussion

In this study we have sequenced and genotyped samples of reported index cases with serologically confirmed acute HAV infection during a two-year period in Amsterdam. Our findings support, what was suggested in our pilot study [9], that there are two distinct high-risk groups: men having sex with men (MSM) and children returning from a country with high HAV endemicity (IMP) and their contacts (FAM/SCH). No transmission between these high-risk groups was documented. Strains found among MSM, all genotype 1A, were not seen in school children; nor were viruses imported by children (IMP) found among MSM. Underreporting prohibits stronger conclusions. Especially among children, many infections occur without symptoms [17]. Also, symptomatic infections might go undiagnosed, and lastly, confirmed cases might go unreported. The Amsterdam Sentinel Project, estimated underreporting of hepatitis A in 1979 at 42% [18]. The national sentinel project found, in 1994 to 1997, underreporting more than 55% in the first two years. But in the second two years, however, the incidence of notifications was higher than sentinel estimates [19]. In Amsterdam, the Public Health Laboratory, also working for general physicians in the area, is incorporated within the Municipal Health Service, and directly reports positive laboratory results to the Division of Public Heath (DPH). Also with other Amsterdam laboratories, administrative arrangements are made to report positive findings directly to the DPH. The incidence of reported hepatitis A cases in Amsterdam exceeds the incidence of reported hepatitis A cases in the rest of the country annually by two to five times. This might partly reflect a true difference, but is attributed to better reporting as well.

Men having sex with men

During the study period, four distinct HAV strains entered the homosexual population. After co-circulating endemically among MSM, the virus seems to disappear after several months or a year, suggesting a reproductive rate just below 1. Through the European research network for rapid detection of food borne outbreaks (QLK1-1999-00594) we found that the MSM2 sequence was identical to strain HAV/SA/10/2000/DE at both the VP1-P2a and the VP3-VP1 regions (Genbank: AY028976 and AY027537). It was isolated four months earlier in Berlin, from tourists returning from Ibiza, Spain, without specific information on homosexual behavior. This throws new light on the interpretation of isolate “2001WK36_UNK-Spain speculated~”. Its homology with MSM strains suggested unreported homosexual activities in the Netherlands. The travel to Spain, including Barcelona, where this strain was also identified, could equally be the source of the infection, with or without homosexual risk behavior.

From MSM not reporting high-risk sexual behavior, we isolated strains identical to the MSM strains. Our molecular data confirm the epidemiological findings in Columbus, Ohio, where, during an epidemic of hepatitis A among MSM, high-risk behavior was no additional risk factor for infection [20]. Our findings also confirm the epidemiological suggestion of Rotterdam, where during an epidemic of hepatitis A among MSM, travel history was subordinate to local risk behavior [21]. From the MSM travelers to Australia and the RSA, we isolated strains that were circulating in the gay scene in Amsterdam, before and after their travel. Two transmissions were speculated to originate from the workplace, where in both instances a homosexual colleague with hepatitis A was speculated as possible source. Molecular data confirmed an MSM connection.

Traveling children

This study shows that, over a two-year period, multiple distinct HAV strains are introduced through children returning from visits to their country of parental origin. Such children transmit the virus to siblings, to Dutch schoolmates and their susceptible Dutch parents. There were 24 new viral introductions by children, and three school clusters, but no ongoing transmission for any of these strains. The control policy (active source and contact tracing, with immunization of possibly exposed contacts), seems to keep the HAV reproductive rate close to 1. In genotype 1A, a child with HAV infection of unknown source (2001WK50_UNK), was index for a cluster related to a primary school (SCH1, Figure 2a). A highly similar strain was isolated the previous year. This strain might have been reintroduced from the same origin in Morocco, as we saw in a cluster in genotype 1B. Genotyping strains from children returning from Morocco in other areas of the Netherlands, showed homology with several of our isolated strains (data not presented). We conclude that regional strains circulate in Morocco and are exported regularly to various parts of our country, and presumably other countries in Europe.

The first import case in genotype 3 (2000WK40_IMP), returned from a family holiday in Spain six weeks prior to illness. The isolated strain suggests a possible relation with Pakistan. The children attend a secondary school, attended also by Pakistani children, but no symptomatic case was reported from this school.

Drug users

Contrary to other areas of the world [22-24], we did not find hepatitis A among drug users. In Amsterdam, hepatitis A was reported in drug users annually up to 1995, but since that year hepatitis A is not confirmed in drug users in Amsterdam, while jaundiced drug users are still referred to our laboratory or Department of Public Health. In recent years hepatitis B, C, occasionally hepatitis E related to

travel, and EBV infections are confirmed.

Vaccination policy

With this study we present strong arguments for two distinct active immunization policies: for MSM and for children who import HAV strains. Regarding the first, the HAV disease burden increases with age [25,26]. The average age of reported cases was 24,6 years, but was 38 years for MSM. Universal vaccination is economically justifiable in areas of intermediate endemicity [27], but the Netherlands overall has low endemicity. Nationwide vaccination is thus infeasible on economic grounds [28], even if justifiable on medical grounds. Without such vaccination as primary prevention, active source and contact tracing is the cornerstone in HAV control. This is adequate intervention for the general population but not for MSM, since in our setting sexual transmission occurs mostly in anonymous settings like darkrooms. Our study shows continued transmission of a single HAV strain among MSM, including MSM not engaging in high-risk behavior, but with virtually no spread outside this group. We conclude that all homosexual men in Amsterdam should be vaccinated against hepatitis A. Since the nationwide vaccination program for groups at high risk for hepatitis B includes MSM [29], we advise to use a combined HBV and HAV vaccine [30]. Data from our study show a HAV reproductive rate just below 1, and suggest elimination of HAV in this group is easy to achieve with only a modest increase in protection rate.

To stop HAV transmission by children who import strains, we advise vaccination before departure for all children going to HAV-endemic areas. Our study shows that current prevention based on voluntary vaccination of children, with coverage below 50%, does not stop introduction of HAV in Amsterdam. The existing secondary prevention strategy, consisting of active tracing and immunizing of contacts, prevents further spread of the virus, but occasional clusters, without symptomatic index continue to occur. In the Netherlands, starting in 2003, all children with one or two parents born in countries of medium or high HBV endemicity, will receive HBV vaccination in the first year of life. This policy includes children originating from Morocco and Pakistan, the countries that were a major source of hepatitis A in Amsterdam. Adding hepatitis A to this program, as a combined hepatitis B and A vaccine [31], would prevent 17/29 (59%) primary HAV import cases during our study period. The percentage secondary cases that might be prevented, can only be speculated. Our data suggest that two of three school clusters were directly related to import from Morocco, as well as several secondary transmissions to household contacts (data not shown). In five Amsterdam cases of unknown transmission, molecular data suggests association with HAV import. The proposed targeted childhood vaccination program, will also reduce cases now classified as unknown. Based on our study, a vaccination program for

all children living in Amsterdam is not required.

Virological typing for public health

The VP3-VP1 and the VP1-P2a regions show similar patterns of clustering (Fig 1a, 1b), but VP3-VP1 shows more variability. The latter can be explained by changes over time due to one or two nucleotide differences (cluster MSM2). The similarity shows that both regions can be used for public health purposes. The more variable region, however, might invoke uncertainties in confirming transmission routes, since natural changes over time can obscure existing true relations. This study further shows that for clarification of transmission patterns of a direct feco-orally transmitted pathogen, molecular typing can supplement epidemiological information, as with sexually transmitted hepatitis B [22] and airborne transmitted tuberculosis [32]. With the present data set, molecular typing could not reveal the transmission route in all cases. Structural collection of strains might further reveal relations that now remain obscure. The European research network for rapid detection of food borne outbreaks is effectively contributing to such data collection. Molecular data have added value in outbreak investigation, either in confirming suspected relations [13,33-39], or, reversibly, in ascertaining, in retrospect, that several different strains were involved in epidemiologically uniform outbreaks [40-43].

However, the major achievement of this study lies in its decisive arguments for targeted vaccination policy. Sound epidemiological studies have assisted in developing differentiated vaccination policies [44,45]. Especially in areas of intermediate HAV endemicity, universal childhood vaccination is warranted. A targeted approach reduces costs, but introduces uncertainties in overall protective efficacy. As the molecular data failed to associate isolates for only seven cases of unknown transmission, we conclude that, in the Netherlands, an area of low endemicity, the addition of the two proposed targeted vaccination strategies could optimally prevent more than 90% of new cases of hepatitis A.

Acknowledgements

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Fecal specimens were obtained from an owl monkey (*Aotus trivirgatus*) inoculated with cell culture-adapted HAV.
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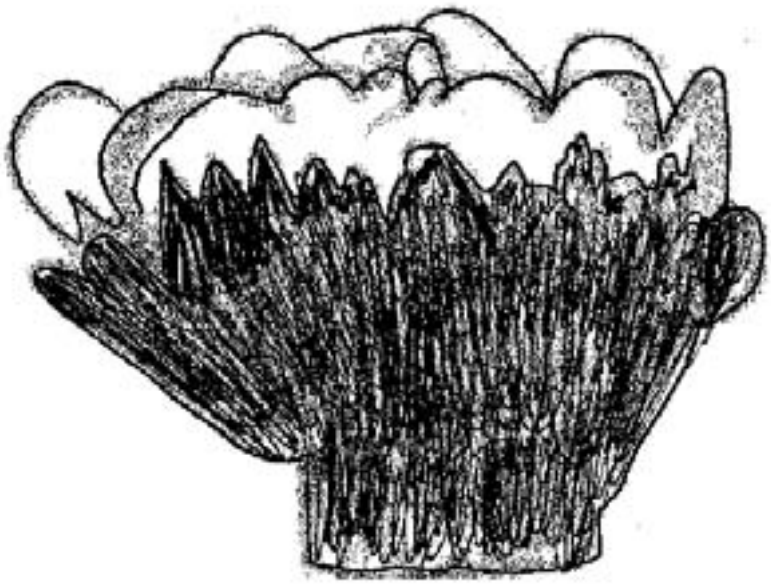
Chapter 9

Discussion and conclusion. Improving hepatitis B and A control in Amsterdam, the Netherlands, an area of low endemicity



The hepdna viruses are closely related to cauliflower mosaic virus and retrovirus.

Abstract

Aims- The seven studies presented in this thesis are aimed at improving viral hepatitis B and A control in Amsterdam and potentially other areas of low endemicity within and outside the Netherlands.

Results- Evaluations in CHAPTER 2 AND 3 show that a program of integrated tracing, screening, tracking and vaccination directed at pregnant mothers (follow-up and referral for treatment if justified), newborns (vaccination and serological follow up), and household contacts (follow-up and referral of carriers and vaccination of susceptibles) is feasible, with good coverage of individuals at risk and good compliance with the vaccination series. Data on vaccination of behavioural risk groups, as described in CHAPTER 4, show that local MHS facilities can reach such groups, although coverage and compliance data may at first be disappointing. Molecular epidemiological data presented in CHAPTER 5 show that IDU transmit hepatitis B to other groups in society, while MSM are much less likely to do so. Horizontal transmission occurs in immigrant populations in Amsterdam. As for hepatitis A, CHAPTER 6 shows that administering IgG does not prevent infection in contacts but can prevent spread of infections introduced by immigrant children; is not effective with MSM. CHAPTERS 7 and 8 show that two separate transmission patterns exist in Amsterdam, with no demonstrable transmission between them.

Conclusions- The MHS approach, integrating antenatal hepatitis B screening with vaccination in communicable disease control is effective and should be implemented nationwide. Alternative local pilot projects with the same goals but perhaps different procedures should be explored as soon as possible. Periodic evaluation is crucial to any approach. If our pilot outreach efforts are implemented nationwide, national publicity and more flexibility for participants increase vaccine uptake. As for hepatitis A, Amsterdam might be better off with a vaccination program for all children with parents originating from areas of intermediate and high endemicity. The need may be obscured by widespread underdiagnosis and reporting. The cost-effectiveness of including hepatitis A vaccine in the hepatitis B programs directed at such children as well as MSM deserves further study.
Molecular epidemiology studies have added value for public health, serving to inform policy decisions, and, if data are collected nationwide, also individual decisions. The spread of HBV and HAV is indicative of the ever smaller world we live in. Problems in remote areas cause problems here and, to protect ourselves, we must assist poor countries. Self-interest can be an added motive for the development of more cooperation.

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Introduction

In this thesis, the results of seven studies are presented regarding the epidemiology and control of viral hepatitis B and A in Amsterdam, The Netherlands, an area of low endemicity for these infections. Three studies describe the evaluations of existing programmes; one is an evaluation of a new program. Three other studies concern the molecular epidemiology of both infections. The majority of the work was done in the Department of Infectious Diseases of the Municipal Health Service Amsterdam. The lessons on the epidemiological situation in Amsterdam are applicable to the situation all over the Netherlands, and might also be beneficial for other countries of low HBV and HAV endemicity. The conclusions per study, discussed at the end of the respective chapters, can be combined to suggest some general conclusions. In this final chapter, an overview is followed by a short general discussion with a conclusion.

Improving existing hepatitis B control

MHS notification

Obligatory notification has an important place in Dutch infectious disease control policy and ideally results in immediate MHS action. The MHS traces contacts and sources, protects exposed susceptibles, and uses notification data to conduct continuous evaluation of the control strategy (surveillance). However, immediate action is difficult because notification always comes late after exposure (after incubation period, patient's delay, doctor's delay and, finally, reporting delay), if it comes at all. For all infectious diseases, there is loss of information leading to underreporting of disease: patients forgo seeing a doctor, doctors forgo confirming a microbiological diagnosis and forgo notifying. Additionally, notification is inherently limited, since most HBV and HAV go unnoticed. Yet, our data from the hepatitis B household-contacts program, directed at contacts of chronically infected women that are identified through the antenatal screening, show that each notification can reveal several infectious, exposed or susceptible individuals. The same goes for cases of acute hepatitis B, as was shown in the outbreak related to a surgeon, where further study of two cases identified 28 infections (1).

An experience that holds true for all MHS facilities is that each notification needs immediate and comprehensive action: immediate to counter the inherent reporting delay and comprehensive to discover the many symptomatic and asymptomatic infections that may lie behind each notification.

A second experience relevant for all MHS facilities is that investigation of contacts is warranted not only for chronically HBV infected women identified in the

antenatal screening but for all carriers reported to the MHS. Starting April 1999, all hepatitis B cases, acute ánd chronic, were to be reported. All sexual and household contacts of chronically infected individuals, especially those from hepatitis B -endemic countries, should be extensively screened, serologically evaluated and protected by vaccination if justified. Since our program (CHAPTER 3) has proven feasible for various MHS facilities, we strongly advise its implementation nationwide and suggest its consideration for other areas of low endemicity.

Improving programs

In CHAPTER 2, we show that enhanced antenatal hepatitis B screening and tracking, as organised by the MHS Amsterdam, is successful. Its framework is efficient and could easily be copied to other MHS facilities in The Netherlands and possibly elsewhere in Europe. The MHS coverage of 97% ranks with the best reported results elsewhere and surpasses the Dutch national program. Crucial to its vaccination coverage and compliance are a centralised enhanced screening and tracking system, a house visit, and a public health nurse responsible for individual cases. Besides, the MHS approach vaccinates as soon as possible after birth, then at month 1 and 6. The National approach integrates neonatal hepatitis B vaccinations into the universal childhood vaccination program, thus vaccinating neonates of chronically infected mothers at month 2, 3, 4, and 11, scheduled with regular childhood vaccines. Its procedures were evaluated in 2001 (2) and while interpretation must be cautious due to administrative shortcomings, coverage is estimated at 90%. Likewise, completion of hepatitis B vaccination series by neonates was reported to be 90%, but for the fourth dose, 84%. Based on this evaluation, the Health Council estimated that each year, 100 children of chronically infected mothers become infected despite the national antenatal and neonatal program. The apparent shortcomings might nearly vanish with better administrative procedures, but every single unvaccinated neonate is a costly missed opportunity, as most will become hepatitis B carriers, at risk for cirrhosis and hepatocellular carcinoma, which require repeated hospital admissions and might result in early death. We agree with the Health Council's suggestion to implement the MHS procedures nationwide (3).

Details

POST VACCINATION SEROLOGICAL EVALUATION OF CHILDREN BORN TO CHRONICALLY INFECTED WOMEN

In the MHS program, we conducted a postvaccination serological evaluation for 521 vaccinated children born to chronically HBV infected mothers, testing anti-

bodies to the hepatitis B surface antigen (antiHBs) at month 7, one month after the third vaccination. Despite administration of appropriate immune prophylaxis, we found three HBsAg-positive cases (0,6%). In subsequent annual check-up, two of the three children lost HBsAg over the years. To calculate the vaccine efficacy against carriership, we thus regarded 3/521 as the incidence in vaccinated neonates and the Council's 270/1000 as incidence for unvaccinated neonates. The efficacy was 97.9%. We were not able to identify a set of criteria for selective post-immunisation screening to predict "insufficient" titres (antiHBs < 10 IU/L). Our data did indicate that male sex and birth weight below 2500 grams were linked to "low" (antiHBs < 100 IU/L). Although antiHBs titres above 10 IU/L can be regarded as protective, it seems prudent always to test anti-HBs in low birth-weight children. For other children, the vaccine efficacy of 97.9% seemed sufficient from the public health point of view. The Health Council, however, saw these neonatal hepatitis B vaccinations as post-exposure prophylaxis and suggested continuing serological evaluation for its individual benefits.
A public health argument for continuation is that analysis of paired sera, from HBsAg positive mother and child, could reveal the emergence of escape mutants that might reduce vaccine efficacy. No such emergence has been reported in the Netherlands, but it needs monitoring; thus we have reintroduced the serological evaluation in MHS Amsterdam, in agreement with the Councils advice.

POST VACCINATION SEROLOGICAL EVALUATION OF HOUSEHOLD CONTACTS OF CHRONICALLY INFECTED WOMEN

For other household contacts, we have considered minimising serological post vaccination evaluation. The percentage of vaccinated individuals reaching sufficient titres after three vaccinations begins to decline at age 30 years (4-6). Our data on 131 adults over 30 years showed 12,2% with antiHBs titre below 10 IU/L. For the age group 30 years and over, evaluation is justified, with revaccinations as required. For those under 30, vaccination results are acceptable for public health purpose: we found only 4,5% antiHBs titres below 10 IU/L. However, vaccinating household contacts protects individuals more than public health. In these situations of continuous exposure, evaluation should still be performed, even in this low age group, to safeguard individual protection.

Improving integration

Tracing hepatitis B carriers in the household (CHAPTER 3) is not only in the interest of the individual but also of public health, because it creates awareness and enables them to take preventive measures, that will reduce the pool of infective individuals, a crucial step in managing the population dynamics of hepatitis B endemicity (7). The tracing and screening of sexual partners, children, and other

household contacts of HBsAg-positive pregnant women should be part of any nation's HBV control program. The MHS approach integrates antenatal screening and neonatal immunization in the routine of communicable disease control. Our data suggest that this has good results in coverage and compliance, both for the newborn child (CHAPTER 2) and other household contacts (CHAPTER 3). At the start of the program in 1989, midwives were not allowed to vaccinate. First vaccination of the newborn child had to be administered by MHS nurses during a house visit soon after birth. Evaluation of comparable programs in the USA (8) suggests that our positive results might be related in part, to this house visit. By amendment of existing regulations in 1998 (3) midwifes are allowed to vaccinate. We propose not to hand over the first vaccination to midwifes, because follow-up of the family remains a MHS responsibility. This first MHS contact to vaccinate at the house of the new-born may be an important motivator for future family compliance. Data from our study on HBV infection rates in household contacts of carrier women, shows that prevalence of markers of infection (antiHBc) increases with age. This indicates ongoing horizontal and sexual transmission, both in children and spouses, and emphasises the need for preventive vaccination of family contacts. We found 57% of contacts susceptible, and these had high compliance for immediate protection, as 91% completed the three-dose series. In Amsterdam, we prefer that antenatal screening, combined with contact tracing, be integrated in the regular communicable disease control programs of the MHS. However, in other areas, where different relationships exist between obstetrical care and child health departments, other solutions might be more productive. Pilot programs tailored to local situations are needed.

Filling the gaps

On request of the Ministry of Health we conducted a pilot program directed at vaccinating groups at high risk for sexual and drugrelated hepatitis B transmission. Over 18 months, an estimated 22% of targeted individuals started the vaccination series. However, while MHS facilities in the Netherlands seem able to enrol high-risk individuals, we must acknowledge that in the same 18 months only 60% of participants completed the vaccine series. Still, vaccinating high-risk behavioural groups is an essential component of a targeted hepatitis B vaccination strategy, as was chosen in this country. More time is needed to improve coverage and compliance. The Ministry of Health acknowledged the potential of our pilot and enabled the MHS Association to co-ordinate nationwide implementation of this new strategy, making relevant funds available for all MHS facilities. Nationwide implementation will enable better publicity and allow participants to complete the vaccination at various locations. In November 2003, one year after its start, preliminary data of this national program show that 11,096 participants

were enrolled, of whom 24% had serological markers of previous infection, and 1,4% markers of chronic infection. Compliance seems acceptable, as 90% of susceptible participants came within two months after the first, for the second dose. It is too early to draw conclusions, but initially the program seems successful. The programs costs will be monitored and included in the mathematical modelling study of the RIVM.

Lessons from molecular typing

IMPORTANCE OF IMPORTATION

The HBsAg prevalence in pregnant women in Amsterdam is in accordance with prevalences found in their various countries of origin. In 1993-98, the overall prevalence for Amsterdam pregnant women was 1.22%, as in 1989-92 (9). National prevalence in antenatal screening was 0.36% in 2000-01 (2), and 0,44% in 1989-92 (10). These figures contrast with the prevalence rate of 0,2% found in the population-based seroprevalence study by the RIVM (11). Data of antenatal screening can not directly be used to estimate for the general population, as women in the reproductive phase are not representative of the general population. However, HBV being sexually transmissible, they are a relevant population. The RIVM included insufficient numbers of participants from areas of intermediate or high endemicity. In Nordic countries outside the Netherlands, seroprevalence increased in the last decade, related to increased influx of chronically infected individuals from areas of high endemicity (12-14). Our data did not show a parallel increase in Amsterdam, possibly because its influx from former Dutch colonies began before that decade. However, molecular epidemiology (CHAPTER 5) shows that, in addition to the vertical transmission, there is horizontal (intrafamilial and sexual) transmission continually in this population. The Mediterranean virus cluster and various incidental exotic genotypes found in our analysis, show that, even with complete coverage by the existing antenatal screening and neonatal vaccination programs, HBV transmission will continue. Our findings are based on only 54 sets of data on patients and the virus isolated from them. Acknowledging this limitation, we calculate that 15% of new cases in Amsterdam occurred through horizontal (including sexual) transmission in a residential population originating from Morocco, a country with intermediate endemicity. The Health Councils advise to vaccinate children with one or both parents originating from an HBV-endemic country (15), would help prevent transmissions in this population that were formerly labeled as possibly healthcare related, household contact and unknown transmission. The Council's advice was adopted by the Ministry and, from March 2003, implemented nationwide.

LIMITATION OF CHILDHOOD PROGRAM

There is one important shortcoming of the newly implemented program for children with background in countries of intermediate or high endemicity, which limits its positive effects. It defines parental origin as “country of birth”, not “country of family origin”, excluding second and third generation parents (born in the Netherlands). Such parents compose one third of the immigrant population (16) and regularly travel with children to the home country. In 2002, this group included approximately 300,000 individuals in our country. Our limited set of data could not quantify the contribution of this theoretical program flaw, to unknown transmissions. However, with increasing numbers of second generation immigrants, this contribution is certain to increase, should be monitored, and added to the next mathematical modelling study. Additional calculations are needed to compare expanded inclusion criteria for the targeted childhood vaccination (country of origin, including second and third generation parents) with the present criteria (country of birth, including only first generation parents).

DRUG USERS

Every year until 1996, acute hepatitis B was confirmed in 2-6 Amsterdam IDU. In the following six years, it was diagnosed only once, suggesting that the hepatitis B problem among IDU is disappearing. We have not studied the reasons of this decreasing incidence, but they might include decreased numbers of susceptibles, as over the years most IDU were already infected. In our country, starting drug users now seldom administer drugs by injection and among users still injecting, new behaviour might also contribute to the decreased incidence. For example, harm reduction programs directed at IDUs were implemented in the late 1980s. Our molecular sequencing data (CHAPTER 5) provide evidence that, despite the decrease in new cases among IDUs, hepatitis B virus is transmitted from IDUs to the non-drug-using heterosexually active population. The hepatitis B vaccination pilot program (CHAPTER 4) and its national implementation includes various outreach programs directed at reaching drug users. They prevent not only new cases in drug users but, by screening all participants, traces chronically infected hepatitis B sources. The latter IDU are the most probable sources for the spread of the virus to the non-drug-using population. This contribution of drug users should be included in the RIVM model for calculating the cost-effectiveness of various vaccination strategies for the Netherlands.

MEN HAVING SEX WITH MEN

Transmission of hepatitis B from MSM to the heterosexual population was detected only once in our small set of data. Transmission from MSM to IDU or vice versa was not seen. For HIV epidemiology, similar results are found in Europe

(17-18), while this exclusive clustering of viral strains according risk groups is not seen in the USA (20-21). Comparable US data regarding HBV are lacking. Protecting MSM is beneficial for their specific group but seems, in Europe, not to contribute in reducing other/non-MSM HBV transmissions. Our data indicate that there is very limited exchange between homosexual and heterosexual groups at high risk for acquiring hepatitis B. This assumption in the mathematical modelling study of hepatitis B is now justified by our data.

TRAVELLERS

In our small set of data, 13% of hepatitis B transmissions originated from travel-related exposure in HBV-endemic countries. Our data suggest that for male travellers that have sex with men, the sexual behavioural risk exceeds the risk posed by travel. The data however confirm that travel is a risk in itself and thus support the recent adjustment of the national guideline indicating hepatitis B vaccination for travellers. The vaccine is now indicated for all travellers with travel duration of three months and over. In case of expected additional risks (occupational, sexual, medical) it is advised irrespective the travel duration.

FILLING THE GAPS

Besides MHS notification with immediate action and notification-based surveillance, the Dutch hepatitis B control policy relies, as described, on a comprehensive targeted vaccination program. The approach now consists of nine programs with various success rates. Addition of universal childhood vaccination was calculated to be not cost-effective, because HBV medical costs are heavily dictated by the hospitalizations of chronic carriers. Their number is not influenced markedly by any vaccination strategy, since carriership is found in persons originating from countries of intermediate or high HBV endemicity, who come to the Netherlands, after being infected in the country of origin. Therefore, in addition to those existing strategies, we propose serologic screening all immigrants from HBV-endemic countries. Those found chronically infected should be offered treatment for their individual benefit and also to minimize the pool of HBV-infectious individuals. Susceptible immigrants should be offered vaccination. This strategy could be tested in a pilot program; its results should be included when analysing the cost-effectiveness of these combined strategies in comparison with universal childhood vaccination.

MOLECULAR AND EPIDEMIOLOGICAL DATA COLLECTION

We have argued that active source and contact tracing for each identified infection, acute or chronic, remains essential in preventing horizontal (including sexual) transmission. However, we acknowledge that since most infections go unno-

ticed, they never come to the attention of the public health professionals. Besides, for over 20% of notified cases, no probable source is identified. We argue that adding molecular virological data (i.e. to study the molecular epidemiology) creates better opportunities to reduce the number of unknown sources and to find weaknesses in the vaccination strategy. Virological typing should be performed on all acute hepatitis B cases in this country and, combined with epidemiological information, the data should be collected in a national data bank, accessible to all MHS facilities. This type of data collection should start as soon as possible.

NEVER ENDING STORY

A final observation is, unfortunately, that, however refined a targeted approach, 100% coverage of all individuals at high risk of acquiring hepatitis B cannot be achieved (CHAPTERS 2, 3 and 4). For one thing, the behavioural risk groups must first display risk behaviour before they can be targeted for vaccination. To improve existing programs (antenatal screening, vaccinating newborns of chronically infected women, with contact tracing and vaccinating as required), expand programs (to behavioural risk groups and children originating from endemic countries), or add new targets (tracing chronically infected immigrants) will not suffice, because the virus adjusts (escape mutants), the population changes (immigration), behaviour changes (sexually and by such customs as tattooing). The societal perception of acceptable risks also changes: There is less acceptance of preventable diseases. Without universal childhood vaccination, public health professionals must continually monitor, evaluate, and adjust the targeted approach. With addition of our proposed strategies, an acceptable level of hepatitis B in the community may be achieved, but for how long? The costs and benefits of universal vaccination clearly must be weighed periodically against the combination of the chosen strategies to see if the latter are still preferable. The negative consequences of introducing a multivalent vaccine, including hepatitis B, into the universal vaccination program seems limited to its costs, since the HBV component is safe, effective, and does not hinder the effectiveness of the other components. A practical solution for the Netherlands might be to implement universal HBV vaccination while continuing the targeted approach for two or three decades, continuing to screen all new immigrants.

Improving hepatitis A control

The Netherlands has gone through a period of epidemiological transition with HAV. It was endemic as a mild childhood disease until probably the Second

World War, as indicated by a 77% seroprevalence of HAV antibodies in persons born before 1945 (23). With increasing hygienic standards, including provision of safe and clean water and sewage disposal, incidence is now low. The result is an increasing number of susceptible adults. As a consequence, hepatitis A is acquired at a later age, leading to more morbidity and mortality. In this epidemiological situation we will see larger community outbreaks related to HAV importation, more hospitalisations and deaths. Given the new safe and effective vaccine, a new policy is required.

Improving notifications

Similar to hepatitis B, notification of MHS remains a cornerstone in Dutch hepatitis A control. Yet, CHAPTER 6 shows that even with early warning and rapid response, the program is doomed to fail repeatedly. Immune globulin might prevent spread, but will not prevent infection. The actual situation is that, with patient's and doctor's delay, and underreporting, notification is inherently late or absent, precluding intervention. Besides, most hepatitis A infections go unnoticed, so prevention efforts reach a minority of infectious individuals. Awareness in healthcare professionals needs improvement; those involved in cure are not always aware of their important preventive position. Laboratory confirmation of a clinical diagnosis might not benefit a patient but can be vital for public health, spurring appropriate action. Besides, unconfirmed diagnoses do not qualify for reporting and thus, opportunities for prevention are missed. This situation frustrates control of HAV and also for other communicable diseases. We advise that MHS facilities get involved in training of healthcare professionals, introducing hepatitis A as a potentially fatal illness for adults and generally raising their public health awareness.

Improving details

VACCINE FOR POST-EXPOSURE PROPHYLAXIS

Based on the findings in Chapter 6, we advise a change from administering immune globulin (IgG) to using vaccine for contacts of reported HAV patients. The latter has a long term benefit for children, as well as for the community, as future introductions are prevented. This conclusion is acknowledged by the National Coordinator Infectious Disease Control (Landelijke Coordinatiestructuur Infectieziektebestrijding, LCI) and adopted in its new guidelines to be published in 2004 (24). Since IgG gives immediate protection, while vaccination takes several days to give protection, the latter might be suboptimal for certain groups. The guideline restricts IgG for individuals at increased risk of serious sequelae of hepatitis A, i.e. those aged 50 and over, or with pre-existing liver disease. Data on age of patients are not presented in CHAPTER 8; however, most strains ware

imported by children. Our data justify the proposed policy of the LCI.

Introducing new programs

INTRODUCING TARGETED VACCINATION APPROACH

Universal HAV vaccination appears not to be cost–effective (25), but no alternative national policy or regulation facilitates childhood vaccination. Since vaccine costs can be a hardship, local policy is sometimes implemented in which MHS facilities or health insurance companies promote vaccination and increase coverage by having vaccination days in early summer to offer low-cost or free vaccine. The coverage of these initiatives is not nationally evaluated, but in Amsterdam such a program achieved coverage estimated at 60% (26). We suggest implementation of a national financial regulation that would enable all immigrant parents to afford HAV vaccine for their children.

INTEGRATING HEPATITIS A IN THE HEPATITIS B PROGRAM

Since a combined safe and effective HBV/HAV vaccine is now available, it should be considered for use in the recently implemented hepatitis B strategy. It could replace the HBV-only vaccine given to all new born children of parents originating from countries of intermediate and high HBV endemicity (15). As shown in Figures 1HBV and 1HAV of the first chapter, target populations for the HBV program include the target children of our proposed HAV control strategy. A study should be conducted to determine if this combined approach is cost–effective.

Lessons from molecular typing

DRUG USERS

In recent years, infections with hepatitis C, occasionally hepatitis E related to travel, and infections with Epstein Bar Virus (EBV) have been confirmed in jaundiced drug users (DU) in Amsterdam. In contrast with the epidemiological situation in other European countries, hepatitis A has not been reported in DU, in Amsterdam, since 1995. We have not studied the causative mechanisms for its disappearance from DU but suggest that most of our DU have acquired immunity as a consequence of previous infections. Equally, there might be social differences among DU in the Netherlands as compared to those of other European countries. Also, the disappearance might be caused by a reduced force of HAV infection, leading to a lower risk of introduction. Since studies so far have been modest in numbers and time frame, HAV might reappear if studies are more comprehensive.

MEN HAVING SEX WITH MEN

The combination of virological typing and epidemiological investigation showed that there is ongoing transmission of HAV among men having sex with men (MSM), once the virus is introduced in this population. However, all introductions die out within several months, compatible with an effective reproductive rate below 1. Our data set revealed no HAV transmission from MSM to others or vice versa.

CHAPTER 6, 7 and 8 show that the usual approach of post-exposure prophylaxis does not work among MSM, as most acquire HAV infection through anonymous contacts, by definition untraceable. If HAV morbidity is to be reduced in this group, the only solution is to vaccinate all MSM. Given the existing HBV control program, targeting all MSM, as described in CHAPTER 4, we suggest a cost-effectiveness study to compare that program with one using the combined HAV/HBV vaccine.

General discussion

Evaluation of communicable disease control interventions

This thesis presents evaluation data of existing and new intervention strategies for HBV and HAV control. New data are used as available, sometimes before publication, in adjusting local and national prevention strategies. MHS facilities should regularly evaluate their communicable disease control activities scientifically, in order to achieve a more evidence-based discipline. This need not necessarily have to be pure, innovative scientific work; a proper evaluation and reporting according to acceptable scientific standards would suffice. As long as such evaluations lag behind, ineffective interventions will waste resources. For example, the currently advised HAV-control intervention in primary schools reporting two independent cases, is to give IgG to all parents and siblings of all pupils. Is this overkill? Society would be better off with regular program evaluations, not only financially but also medically, as all interventions have side effects. For example, the effects of post-exposure chemoprophylaxis for pertussis or streptococcal group A disease are insufficiently studied, but no public health physician forgoes the use of antibiotics. Such interventions are not evidence-based. Since invasive infectious occur rarely, MHS facilities should join forces to collect sufficient data within a short time. In general, working methods would improve if all interventions are regarded as projects, with evaluation as part of the assignment. Our evaluation studies on the antenatal HBV screening and neonatal immunization program (CHAPTER 2) and the household contact program (CHAPTER 3) serve as examples of this approach. The results are used for improving the Am-

sterdam program and should have bearing in other areas. Moreover, they can be used as input for economic cost-effectiveness studies on the national level. Likewise for HAV, our evaluation of the post-exposure prophylaxis (CHAPTER 7) served to improve the national guidelines. As long as scientific evidence is missing, too many communicable disease control interventions rely on circumstantial evidence.

Molecular epidemiology

POWERFUL TOOL

Molecular epidemiology, that is, the combination of molecular data on the agent and the epidemiological data on the patient, is a powerful tool for public health. This is shown for many infectious diseases, from studies of tuberculosis (27; 28), to our studies of HBV/HAV in Amsterdam. If data originating from a wider area can be included for making possible connections, the output for tracing sources will increase. However, data of molecular virology should always be interpreted with caution, because technical imperfections sometimes suggest an invalid connection, or obscure valid connections.

SURVEILLANCE

Epidemiological surveillance remains an essential part of evaluative research and leaves a high percentage sources “unknown”. In our studies, the contribution of virological typing was not decisive for all individual cases. However, we used clustering of isolates from patients with source “unknown” to conclude that the Mediterranean HBV is incidentally imported but also transmitted within the Mediterranean population, and sometimes to others. Epidemiological information in the worldwide gene bank is insufficient for a local public health purpose. Linking individuals to possible sources or common exposures requires a national or, better yet, a European data bank. Collection of this type of data for HAV was started recently by the “Food borne Viruses in Europe network” (29). For a Dutch HBV databank, the first step has been taken by RIVM, MHS Amsterdam and Rotterdam. The combination of viral typing and epidemiological data will help to find previously undetected common exposures and, by doing so, will point to other co-exposed persons, in time to vaccinate susceptibles.

TECHNICAL UNCERTAINTIES

Typing systems are still in development. Systems might be too refined, allowing natural occurring genetic or phenotypic changes to obscure transmission patterns. On the other hand, systems might be too general to differentiate and reveal transmissions. For HBV, a highly conserved virus, a reasonable consensus

has now emerged to sequence the complete S-gene, but not the complete viral genome. This seems to satisfy public health needs. For HAV, the recent development is to use only a small area in most studies (VP1-2a), though there is a tendency to attempt using larger areas, even the complete genome. We used two regions that showed similar patterns of clustering, but the second region (VP3-VP1) showed more variability and might impede confirming transmission routes, since natural changes over time can obscure true relationships. By combining the data from the two regions, effective typing for public health purposes was achieved.
However, the continual confrontation of laboratory and epidemiological data occasionally produced irreconcilable results. This conflicting data led us to repeated analysis of source material and repeated computer calculations for generating phylogenetic trees. New data generated new interpretations. Molecular epidemiological studies thus have to be interpreted with caution. Only repeated observations in independent studies can confirm suggested theories.

Global aspects

There is a striking global similarity between HBV and HAV endemicity (Figure 1 of CHAPTER 1), but HBV and HAV epidemiological patterns differ and thus control strategies differ.

Hepatitis B
Worldwide, most HBV infections occur in children, at birth or in their first six years. These early infections can cause disease and death three decades or more later in life. In areas of intermediate and high endemicity, universal childhood hepatitis B vaccination is the best public health strategy, preferably integrated in the routine vaccination program. In 1992, WHO recommended introduction of universal vaccination for all countries by December 1997; and by May 2003, 151 of 192 member states comply (30). Costs pose a problem, not only for the vaccine but also for delivery. Universal vaccination needs a solid public health infrastructure, not available in many countries, especially those of high endemicity that need the vaccination most. If a country is not able to provide routine vaccinations to the majority of its children, it is of little use to add hepatitis B vaccination.
The burden of disease in countries that are rich and in general of low endemicity mainly originates from HBV importation by chronic carriers (12-14). This goes especially for the Nordic countries (Scandinavia, Ireland, UK, The Netherlands), where universal vaccination is usually not cost-efficient (31; 32). In the deprived

areas, universal vaccination would save costs and lives, but resources and infrastructure are lacking.
Despite the highly specific targeted vaccination approach in the Netherlands, the number of chronic carriers, with consequent morbidity and mortality, is not reduced. This can be explained by HBV importation by existing carriers from countries of high endemicity that become Dutch residents. These chronically infectious immigrants will continue to generate HBV-related costs later in their lives. The situation shows that the mobility of populations makes our world, indeed, into a global village, and that problems elsewhere (remote poor areas of high HBV endemicity) must be solved there, in order to improve the health here (rich areas of low endemicity).
Dutch HBV epidemiology is dominated by global HBV epidemiology. It thus seems a practical strategy for rich countries of low endemicity to support poor countries of high endemicity, so that the latter can provide universal HBV vaccination for their children.

Hepatitis A
In areas of high HAV endemicity where seroprevalence is over 90%, hepatitis A is a childhood enteric infection. Most children are infected before the age of 10; sanitation and hygiene would reduce the infection rate and provision of clean water and proper waste management would solve the problem. In areas without such amenities, HAV vaccination programs would be a waste of money, as in those circumstances it is preferable to acquire the disease at a very young age. In other areas, where standards of living are improving, the age of first HAV infection rises and communitywide epidemics occur (33). In those areas, universal HAV vaccination might become an attractive, relatively cost-effective intervention. Only when we have reached this stage all over the world, should we discuss hepatitis A as target for eradication. Again, as with HBV, HAV shows that infectious diseases are global: the rich world has the facilities and money to improve health and reduce transmission, but poor countries do not. Rich people travel to poor countries for adventure and acquire disease there; poor people from high endemic countries travel to rich countries for work and wealth. This global mobility imports virus to the general population in rich areas.

Consequences
Until eradication of HBV/HAV, the rich world lives with two vaccine-preventable diseases, which cause increasing morbidity. There is a shortage of IgG and usage shows failure and safety problems. Vaccination strategies in the rich world, targeted or universal, will use economic and infrastructural assets, which could instead be used to assist the poorer world to solve their HBV and HAV problems

and reduce the burden of disease in both worlds.

Conclusion

Routine evaluation of public health strategies by analysis of regularly collected data or by using new molecular techniques is essential to maximise efficacy and minimise waste of resources. Specifically for the control of HBV/HAV the present development of a safe and effective combined vaccine, enables a relatively cheap and effective adjustment in the Dutch control policy for the immediate future. In the long run, universal vaccination against both diseases might be justified even in this area of low endemicity. The ultimate solution, however, would be to assist countries of intermediate and high endemicity to control their hepatitis problems by provision of unlimited supply of clean injection needles and condoms (HBV) and clean drinking water (HAV) and by introducing vaccination once such basic needs are met.

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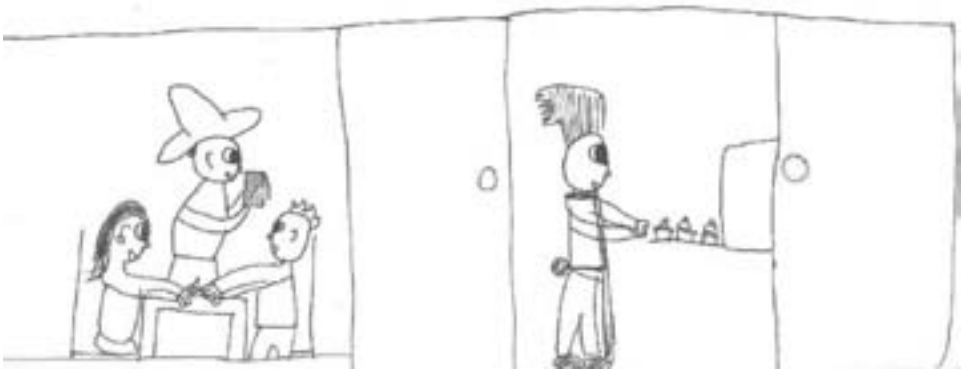
MONACA, Pennsylvania: Hepatitis A Outbreak Toll Reaches 500
The number of people infected in a hepatitis A outbreak linked to a Mexican restaurant has exceeded 500 and is likely to continue rising for another week, state Health Department officials said. Three people infected with the virus have died, and about 8500 people have received shots immune globulin because of the outbreak linked to a Chi-Chi's Mexican restaurant at the Beaver Valley Mall, about 25 miles northwest of Pittsburgh. As of Fri 21 Nov 2003, 575 cases of hepatitis A have been confirmed in the outbreak. Contaminated green onions, chopped up raw in salsa that was served free to every table at a Chi-Chi's restaurant almost certainly caused the outbreak. Food preparation at the restaurant in Monaca, Pennsylvania, amplified the problem, health experts said.
New York Times, Sat 22 Nov 2003 [edited]



In April and May 1986 a large common-source epidemic of hepatitis occurred in Ogemaw County, Michigan. Investigation documented that the source of the outbreak was a local bakery and that glazed and iced pastries served as the common vehicle. In the first two weeks of April 1968 two cases were reported: one was in an assistant at the bakery in West Branch, the largest city in the county. Then, over a one-month period from April 28 to May 26, 61 more cases of hepatitis occurred in Ogemaw County residents. It is strongly suspected that the majority of exposures to contaminated pastries were on Friday, April 5.
Stephen C. Schoenbaum, Ophelia Baker, Zdenek Jezek. Common-source epidemic of hepatitis duet o glazed and iced pastries. American Journal of Epidemiology 1976, vol 104, no 1. 74-80.[Edited]

Summary

CHAPTER 1 offers an introduction on the epidemiology and control of hepatitis B and A in Amsterdam and the Netherlands. In both infections, most cases go unnoticed. The incidence of new HBV infections can be estimated at 2,250 annually in the Netherlands, of which 750 are symptomatic (4.8/100.000/year). The annual number of hospital admissions for hepatitis B is at least 350, and the number of chronically infected individuals exceeds 59,000 (370/100,000), leading to an annual mortality of approximately 60 (related to 1/1,000 carriers/year). For one in every five acute illnesses reported, no probable source can be found. There are doubts about the effect of the targeted vaccination strategy that was decided upon in the Netherlands since important data are lacking on the effectiveness of the antenatal HBV screening, on immunization of newborns of HBsAg-positive women, on vaccination of household contacts of HBsAg positive individuals, and on transmission to and from various high-risk groups. In addition, the targeted vaccination does not reach groups at high risk for hepatitis B through sexual and drug-related behaviour. Data on which to base calculations for the cost-effectiveness of universal vaccination are lacking. With regard to hepatitis A, the number of annual infections can be estimated at 3,000, leading to 100 hospital admissions and 1-5 deaths. A new vaccine is on the market but is underused. Unprotected children at high risk of acquiring hepatitis A while visiting their country of parental origin continue to import the virus, possibly causing secondary and tertiary spread in the community. Data on the relative importance of these introductions, as compared to what may be local endemic transmission, are lacking. Consequently, a coherent vaccination policy cannot be developed, and various local vaccination initiatives are not sufficiently supported with national guidelines and regulations. By evaluation of existing programs, addition of new programs, and especially by using molecular epidemiology, we try to illuminate the unknown aspects of HBV/HAV epidemiology that could assist policy decisions.

CHAPTER 2 evaluates the antenatal hepatitis B screening and neonatal immunization program in Amsterdam. The enhanced program differs from others in the Netherlands in active tracing and tracking HBsAg-positive women with their families and early administration of vaccine to the newborn by the MHS, as opposed to passive registration and vaccination at month 2 by the Child Health Clinics. This enhancement required adding one half-time person, yet was relatively successful. In the period 1993-98, 691 HBsAg-positive pregnant mothers were reported. The coverage of the screening was calculated at 97%. HBsAg-prevalence was highest in women from Ghana and South-East Asia, and

lowest in women of Dutch background. Hepatitis B immune globulin (HBIG) was administered within 24 hours to 95.9% of the neonates, of whom 99.7% completed the vaccination series. Six weeks after the third vaccination, 85% of children had antiHBs titres of ≥ 100 IU/L; in 12% had titers 10-100 IU/L; 3% had titers < 10 IU/L. Of 521, 3 initially had HBsAg. Low birth weight (OR 3.77), male-sex (OR 1.64), HBV-endemic country of origin were predictors of low postvaccination titers. The program could be implemented in other parts of the country and possibly also in other areas of low endemicity.

CHAPTER 3 evaluates Amsterdam's program directed at tracing and immunising household and sexual contacts of expectant women who are identified in the antenatal screening program. It is integrated in the work of the Department of Infectious Diseases of the MHS. Contacts are traced and tested for serological markers of previous infection, and vaccination is offered to susceptible contacts. Those chronically infected are counselled and referred for treatment if justified. In a period of eight years (1992-1999), 738 women newly identified as testing HBsAg-positive reported 1219 contacts; 1100 (90.4%) contacts were tested and 476 (43%) had serological markers of previous infection, of whom 119 (25%) were infectious. Of 603 contacts to be vaccinated, 568 (94%) completed the series. Country of origin was an independent predictor of contact participation and completion of the series. Postvaccination titres for antibodies against the surface antigen were below 10 IU/L in 4.5% of contacts under 30 and in 12.2% of those over 30. This integrated approach leads to finding individuals at high risk for acquiring hepatitis B, who are motivated for vaccination. Tracing and immunising susceptible contacts of women screened as HBsAg-positive should be integral to any nation's HBV control program. Vaccinating contacts serves predominantly individual protection but provides collateral public health benefits. Though not always needed for public health, the serological post-vaccination evaluation of contacts should be continued to ensure individual protection.

CHAPTER 4 describes the organization and evaluation of a two-year pilot project, directed at vaccinating high-risk groups for sexual and drug-related HBV transmission. Funded by the Ministry of Health, seven participating MHS-facilities offered hepatitis B vaccination without cost to MSM, DU, and heterosexuals with multiple partners, including commercial sex-workers. Participants were enrolled during the first 18 months of the project (October 1998 – May 2000). Second and third vaccinations were given up to October 2000. Three of the seven MHS facilities, designated as control, used flyers only to invite people to come for free vaccination at the regular opening hours of the MHS. Four facilities, designated as intervention areas, used extra recruitment strategies,

either by intermediary caretakers or by the MHS staff: methadone and STD-clinics included the vaccinations in their standard operating procedure; certain MHS facilities deployed opinion and peer leaders to recruit; others used peer DU; outreaching vaccination was carried out in non-medical low-threshold places; intermediaries and DU were offered a financial incentive. In 18 months, 13,808 people from high-risk groups entered the program, with uptake of 63% of the targeted population in the intervention areas compared to 23% in the control areas. The enrolment of DU remained far behind expectation (19% in intervention regions, 4% in control regions). The enrolment of the heterosexual population (64%) was satisfactory, due partly to inclusion of vaccination in the standard operating procedure of the Amsterdam STD-clinic, and also to multiple outreach efforts. There were regional differences in the success and failure of various recruitment strategies, but clearly MHS facilities in the Netherlands can reach and enrol high-risk individuals for hepatitis B vaccination if sufficient resources are supplied; more time is needed to implement the program, especially for hard drug users. The flexible targeted approach is feasible; successful strategies in one region are not uniformly transferable to other regions. The vaccine coverage of the targeted population was disappointing, because insufficient numbers of high-risk individuals were reached to protect their population against HBV. However, even with universal vaccination, these high-risk groups would need special attention. Our experience with the various strategies will be useful in other MHS facilities. The most successful strategies might be applied elsewhere in the industrialised world.

CHAPTER 5 describes a retrospective DNA sequencing study of isolates from stored sera from reported primary cases of acute hepatitis B infection in Amsterdam. Cases were classified according to risk behavior, as determined in interviews. Of available sera, a selected region of HBV-DNA was amplified and sequenced. The nucleotide alignments were subjected to phylogenetic tree analysis to determine how the strains clustered. Over a six-year period (1992-1997), the strains of 54 isolates could be typed: 26% of the 204 reported primary cases. The strains clustered in five genotypes: A, C, D, E and F. In genotype A, we identified a cluster related to MSM. In genotype D, two subclusters could be identified, one related to IDU and another related to the Moroccan population in Amsterdam. The remaining strains showed a high genetic variability within three different genotypes: F, E and C. Of the 14 identical isolates in the “MSM cluster”, one was isolated from a heterosexual female. Of the 14 identical strains in the “IDU strain”`, six were from non-drug using heterosexual active individuals. In the cluster of 12 isolates related to HBV-endemic areas, probable modes of transmission were varied. Our analysis indicates that the targeted prevention strategy

in The Netherlands fails to stop HBV transmission from chronically infected individuals originating from HBV-endemic countries and also from IDU to the heterosexually active population. In general, sequence analysis provides important insight into the spread of hepatitis B among various high-risk groups.

CHAPTER 6 concerns hepatitis A and evaluates the current hepatitis A control policy regarding household contacts of acute hepatitis A patients between 1996 and 2000 inclusive. The policy is to trace and protect all household contacts with IgG. We analysed the characteristics and serological outcome of contacts, inviting all susceptibles for interview and retesting 6 weeks after receiving IgG to analyse its actual effect in preventing disease and infection. Of the 569 index patients, we traced and analysed data of 1242 contacts. Over 50% (672) of contacts were HAV-immune by previous infection. Of the remaining 570, 161 (28.2%) had a concurrent HAV-infection, of whom 47% showed no signs or symptoms of disease (75/161); 409 susceptible contacts received IgG. Of these, 186/409 (45%) came back after 6 weeks, and we identified serological markers of recent infection in 64 (34%). Of these persons, only 12 could report signs or symptoms of disease in the weeks after the IgG administration. This correlates with 82% silently infectious contacts. However, no tertiary cases were reported. We thus conclude that IgG does not protect all contacts from HAV infection, but attenuates symptoms. It also seems to reduce further HAV transmission.

CHAPTER 7 presents epidemiological and virological data, collected in 1997/1998, from faecal samples of 33 persons with acute symptomatic serologically confirmed hepatitis A infection. In 8 cases, serial stool samples were available. Performance of nested RT-PCR targeting the VP3-VP1 and VP1-P2a regions followed by sequence analysis established the duration of fecal HAV RNA excretion in stool and the epidemiological molecular relationships among patients. Samples of 31 patients were RT-PCR positive, of which 24 were positive for both regions. Fecal HAV shedding was found to occur for at least 33 days after onset of symptoms, which was the longest-time span tested. Sequencing showed that the hepatitis A virus subgenotype circulating among persons of Moroccan descent (type IB) was not the subgenotype circulating among Dutch MSM (type IA). We concluded that molecular epidemiology for hepatitis A can give additional information on transmission patterns in this area of low endemicity. If HAV subgenotype 1A, is confirmed to be endemic among MSM in the Netherlands, this finding should have consequences for the vaccination strategy. We therefore arranged for a comprehensive molecular epidemiology study.

CHAPTER 8 presents data of a viral sequencing study, performed on samples representing all primary cases of acute HAV infection reported over two years in Amsterdam (2000-2002). Again, as described in the previous chapter, two regions of HAV RNA were amplified and sequenced and used for phylogenetic analysis. Of all reported 156 index cases, strains of 104 isolates (66%) could be isolated and sequenced. The strains clustered into three genotypes: 1A, 1B and 3. Two separate transmission cycles, without mutual interrelation, were revealed. In genotype 1A, four clusters were related to MSM, and a fifth related to a virus imported from Morocco. One of the MSM clusters was probably related to importation from Spain. In genotype 1B, six small clusters were directly related to Moroccan import. In genotype 3, cases were related to imports from Pakistan. Our analysis indicated that, to stop HAV transmission in Amsterdam, the entire homosexual population should be vaccinated, as well as travelers to HAV-endemic countries, especially children. The data indicated that prevention strategies need not include vaccination of all children living in Amsterdam. Collecting viral molecular data and patients' epidemiological data can assist in tracing hitherto unnoticed connections. It is advisable for European public health researchers to combine forces, as is done in the European Working Party on Food borne viruses, to collect molecular epidemiological data for a European data base.

CHAPTER 9 summarizes the findings of the previous chapters and adds some general conclusions. The approach of integrating antenatal hepatitis B screening into communicable disease control, with extension to household contacts, is effective and should be implemented nationwide. Alternative local pilot projects, with the same goals but various procedures, should be explored as soon as possible. Evaluation has to be an integral part of all approaches to control all types of communicable diseases. Drug users are a source of hepatitis B to non-drug-users, but MSM seem not to transmit the virus to others. As the MHS outreach efforts are implemented nationwide, wider publicity and more flexibility for participants will enable increased vaccine uptake. As for hepatitis A, children importing the virus and their contacts might be better off with vaccination. The cost-effectiveness of including HAV vaccine in the HBV programs directed at MSM and children originating from countries of intermediate and high endemicity should be further studied.
Molecular epidemiology studies have significant value for public health; data can support policy decisions, and in the future, if collected nationwide or, better, at the European level, data can benefit the individual by confirming hitherto unknown connections. Infectious diseases like viral hepatitis B and A, are indicative of the ever smaller world we live in: problems far away cause problems at the

doorstep. It is in our own interest to contribute to infectious disease control in countries far away as well as at home.

Samenvatting (summary in Dutch)

Dit proefschrift omvat zeven onderzoeken naar het effect van bestrijdingsprogramma's van hepatitis B en hepatitis A (HBV/HAV) in Amsterdam en Nederland. De bevindingen kunnen helpen om de bestrijding van HBV/HAV in Nederland te verbeteren, en zijn ook bruikbaar voor andere regio's waar HBV/HAV weinig voorkomen.

HOOFDSTUK 1 beschrijft wat er tot nu toe bekend is over de ziektelast, de epidemiologie van HBV/HAV en welke bestrijdingsstrategieën in Amsterdam en Nederland zijn ingezet. Voor beide infecties geldt dat de meeste zonder verschijnselen verlopen. Het jaarlijks aantal nieuwe HBV infecties wordt in Nederland geschat op 2.250, waarvan 750 met verschijnselen (4.8/100.000/jaar). Het jaarlijks aantal ziekenhuisopnamen voor HBV bedraagt minstens 350, en het aantal Nederlandse chronisch geïnfecteerde HBV dragers moet meer dan 59.000 zijn (370/100.000 inwoners). Chronisch dragerschap kan na decennia leiden tot ernstige leverschade en soms leverkanker. Het geschatte aantal dragers zou overeenkomen met jaarlijks ongeveer 60 sterfgevallen (sterfte is 1/1.000 dragers per jaar). Nederland heeft er niet voor gekozen vaccinatie tegen HBV aan alle kinderen aan te bieden, maar zet in op gerichte vaccinatie van risicogroepen en brononderzoek bij ieder gemeld geval. Bij één van iedere vijf dergelijke gevallen kan geen vermoedelijke bron worden gevonden. Er zijn dus "onzichtbare" besmettelijke personen die HBV verspreiden en het is onduidelijk welke groepen daar de grootste bijdragen leveren.
Omdat er na 1992 geen kwantitatieve evaluaties zijn uitgevoerd, is het onbekend of en hoe effectief de algemene programma's zijn zoals zwangeren-screening, immunisatie van pasgeborenen van HBsAg-positieve moeders, en hun gezinscontacten. Bovendien is de risicogroepenbenadering niet van toepassing geweest op bepaalde groepen zoals mannen die sex hebben met mannen (MSM), druggebruikers, en personen met veel wisselende sexuele contacten, waaronder ook prostituées. Er is dus onvoldoende inzicht in de verspreiding van HBV tussen de verschillende risicogroepen en het effect van het huidige beleid. Er zijn ook onvoldoende gegevens beschikbaar voor een kosten-effectiviteitsstudie die universele vaccinatie moet vergelijken met het huidige HBV-preventiebeleid.
Voor wat betreft HAV wordt het jaarlijks aantal nieuwe infecties geschat op 3.000, met 100 ziekenhuisopname en 1-5 doden. Er is een relatief nieuw, veilig en effectief vaccin, maar dit wordt weinig gebruikt. HAV wordt jaarlijks geïmporteerd door ongevaccineerde vatbare kinderen, die in de zomer hun land van herkomst bezoeken, met daarna mogelijk ook verspreiding in Nederland (secundaire en tertiaire gevallen). Het aandeel van deze virusimport in de totale HAV-

verspreiding is onbekend. Mede hierdoor kan geen vaccinatieprogramma worden opgezet en verlaten we ons op locale initiatieven om HAV-vaccinatie tegen verlaagd tarief, of gratis, aan te bieden aan mensen die met hun kinderen naar hun vaderland op vakantie gaan. Door bestaande programma's te evalueren en gebruik te maken van nieuwe moleculair epidemiologische technieken, hebben we geprobeerd nieuw inzicht te verschaffen in de verspreiding en daaruit volgende optimale beheersingsstrategie van HBV/HAV.

HOOFDSTUK 2 is de evaluatie van de HBV-zwangerenscreening en immunisatie van pasgeborenen door de GG&GD Amsterdam. Van 1993 tot 1998 zijn 691 HBsAg-positieve zwangeren opgespoord, een bereik van 97%. HBV-dragerschap (HBsAg-prevalentie) is hoger bij vrouwen van Ghanese en Zuid-Oost Aziatische origine, en laag bij vrouwen met een Nederlandse achtergrond. Passieve antistoffen tegen HBV (HBIg) werden bij 95,9% van de pasgeborenen binnen 24 uur na de geboorte toegediend; in totaal voltooide 99,7% van de zuigelingen de vaccinatieserie volgens schema. Bij serologische nacontrole, zes weken na de laatste vaccinatie, was bij 85% de antiHBs titer $\geq$ 100 IU/L; bij 12% was deze 10-100 IU/L; en 3% had een titer < 10 IU/L, en bij 3/521 kinderen was ook antigeen aantoonbaar, een teken voortgaande infectie (HBsAg). Laag geboortegewicht (OR 3.77), mannelijk geslacht (OR 1.64) en land van herkomst van de ouders, waren onafhankelijk geassocieerd met een lage titer na volledige vaccinatie. Het programma steekt qua bereik en trouw in afmaken van de vaccinatieserie, gunstig af bij vergelijkbare programma's elders in de wereld. Het programma wordt centraal gecoördineerd door één half-tijds verpleegkundige en is daarmee relatief goedkoop. Het programma zou ook elders in Nederland goed kunnen werken, en is wellicht ook bruikbaar buiten Nederland in welvarende landen met weinig hepatitis B, waar zwangerenscreening essentieel is. Proefprojecten zouden moeten uitwijzen of de organisatie overal hetzelfde kan worden uitgevoerd, of dat kleine organisatorische aanpassingen tot lokale verbeteringen leiden.

HOODSTUK 3 schetst de uitkomsten van de evaluatie van het contactonderzoek, dat bij iedere melding van een HBsAg-positieve zwangere wordt verricht door de GG&GD. In Amsterdam wordt de zwangerenscreening gecoördineerd door de afdeling Infectieziekten van de GG&GD, die ook het contactonderzoek begeleidt. Gezinsleden, (sex)partners en andere huisgenoten worden opgespoord en uitgenodigd om op de GG&GD serologisch onderzoek te laten doen naar hun HBV-status. Chronisch geïnfecteerden (dragers) die zo opgespoord worden, krijgen informatie over de ziekte en mogelijkheden om verspreiding tegen te gaan, en worden zonodig voor behandeling doorverwezen; vatba-

ren wordt vaccinatie aangeboden. Mensen die al beschermd blijken te zijn door eerdere infectie, worden uit begeleiding ontslagen. Gedurende acht jaar (1992-1999) zijn 738 nieuwe HBsAg-positieve zwangeren via de screening opgespoord, die 1219 contacten opgaven; 1100 (90.4%) contacten kwamen voor serologisch onderzoek; 476 (43%) bleken in het verleden al geïnfecteerd waarvan 119 (25%) chronisch. Van de 603 vatbare contacten maakten 568 (94%) de vaccinatieserie af. Land van herkomst was gerelateerd aan deelname en aan het afmaken van de vaccinatieserie. Van de volledig gevaccineerde contacten onder de 30 jaar, had 4,5% zes weken na de laatste vaccinatie een onvoldoende antiHBs-titer <10 IU/L, en dat was 12,2% bij deelnemers boven de 30. Omdat de vaccinatie vooral individuele bescherming biedt, met beperkt effect voor de volksgezondheid, is verzekering van individuele bescherming gewenst bij deze personen die voortdurend blootgesteld zijn aan besmettelijke huisgenoten (de index zwangere), ook bij de gevonden gunstige beschermingspercentages. Met deze aanvulling op de HBV-zwangerenscreening spoort de GG&GD veel risicopersonen op, die een hoge motivatie hebben om de vaccinatieserie te beginnen en af te maken. Dit soort contactonderzoek hoort bij alle GGD'en en in alle landen met een HBV-zwangenscreening deel uit te maken van basale de HBV-bestrijding.

HOOFDSTUK 4 beschrijft de opzet en uitvoering van een proefproject gericht op het vaccineren van groepen personen met verhoogd risico op hepatitis B. Het ministerie van Volksgezondheid, Welzijn en Sport heeft dit proefproject geïnitieerd en financieel mogelijk gemaakt om uit te vinden of GGD'en in staat zijn mannen die sex hebben met mannen (MSM), druggebruikers en mensen met veel wisselende sexuele partners, waaronder ook prostituées, te bereiken voor HBV-vaccinatie. Zeven GGD'en boden HBV-vaccinatie gedurende 18 maanden gratis aan (oktober 1998 – mei 2000) en de vaccinatieserie kon tot oktober 2000 afgemaakt worden. Drie GGD'en deelden folders uit met informatie, achtergronden, nut en noodzaak van vaccinatie, waarin mensen geadviseerd werd om op bepaalde tijden naar de GGD te komen voor de gratis vaccinatie (passief aanbod). Vier GGD'en hebben extra activiteiten ontwikkeld om de bedoelde risicogroepen te bereiken (actief aanbod). Afhankelijk van de plaatselijke omstandigheden werd gevaccineerd door de GGD of door andere medische instellingen of personen, zoals huisarts, methadonpost, prostitutieproject, of SOA-poli; GGD'en maakte gebruik van sleutelfiguren om deelnemers te bereiken; bij druggebruikers is een Tupperware-methode geprobeerd, waarbij een deelnemer wordt beloond als hij anderen tot vaccinatie kan brengen; vaccinatie werd verricht "op locatie": niet-medische instellingen en plaatsen zoals bijvoorbeeld bordelen, spuitomruilplaatsen en anonieme homo-ontmoetingsplaatsen. Gedurende de 18 maanden zijn 13.808 personen met vaccinatie begonnen; dit

was 23% van de doelstelling bij GGD'en met passief aanbod, en 63% van de doelstelling van GGD'en met actief aanbod. De deelname van druggebruikers was teleurstellend laag (4% bij passief aanbod, 19% bij actief), hetgeen voor een groot deel te wijten was aan het traag op gang komen van een structureel aanbod via instellingen voor verslavingshulp. MSM werden redelijk goed bereikt (64%), hetgeen voor een deel te danken was aan de Amsterdamse SOA-poli, waar HBV-vaccinatie aan iedereen met een SOA werd aangeboden, en voor een deel door de zeer gevarieerde aanpak van GGD'en, naar gelang de lokale mogelijkheden. Er waren grote verschillen tussen de regio's in sterke en zwakke strategieën, waardoor geen universele succesformule kon worden geformuleerd, maar het project leverde wel een breed scala aan mogelijke benaderingsstrategieën op waar alle GGD'en uit kunnen kiezen wat het beste is voor hun regio. GGD'en bleken in staat deze risicogroepen tot op zekere hoogte te bereiken voor HBV-vaccinatie, mits daarvoor voldoende middelen worden vrijgemaakt. Er bleek meer tijd nodig om alle groepen te bereiken, met name om vaccinatie beschikbaar te stellen aan druggebruikers. Het berekende bereik van de som van alle risicogroepen was 6% bij passief aanbod en 22% bij actief aanbod. Landelijke invoering biedt meer mogelijkheden tot publiciteit en flexibiliteit voor mobiele deelnemers, waardoor het bereik zal stijgen. Niettemin is het onwaarschijnlijk dat het bereik ooit zo hoog zal worden dat HBV overdracht stopt. De risicobenadering kan, per definitie, pas personen bereiken nadat ze al risico gelopen hebben, en blijft altijd achter de feiten aanlopen. Desondanks moet maximaal geprobeerd worden deze groepen te bereiken, ook nadat een algemene vaccinatie tegen HBV is ingevoerd, totdat iedereen die risicogedrag gaat vertonen reeds in de kindertijd gevaccineerd is. Risicogroepen vaccinatie zal nog enkele decennia nodig blijven. De in dit proefproject opgedane ervaringen zijn bruikbaar in heel Nederland en ook in andere landen, waar deze risicogroepen nog niet beschermd zijn door vaccinatie. Het Ministerie heeft voor landelijke invoering gekozen en vanaf mei 2003 kunnen alle personen uit deze risicogroepen gratis HBV vaccinatie verkrijgen op alle GGD'en in het land.

HOODSTUK 5 toont de bevindingen van het moleculair epidemiologisch onderzoek in Amsterdam op basis van zes jaar HBV-meldingen aan de GG&GD (1992-97). In dit retrospectieve onderzoek zijn gegevens van 54 patiënten gekoppeld aan bevindingen van genotypering van het bij hen gevonden HBV. Mensen die gemeld worden met een acute hepatitis B infectie worden op basis van een na anamnese ingedeeld naar vermoedelijke bron van overdracht: mannen die sex hebben met mannen (MSM), heterosexuele overdracht, druggebruikers, huisgenoten van besmettelijke personen, medische ingrepen, en "onbe-

kend". Bij 54/204 (26%) in de studieperiode gemelde patiënten, kon DNA-genotypering verricht worden, door een specifiek deel van het HBV-DNA te vermeerderen en tot op de nucleotide-volgorde te ontleden. Van de DNA-gegevens is een phylogenetische boom (afstammingsboom) gemaakt. De geïdentificeerde stammen behoorden tot vijf genotypen: A, C, D, E en F. In genotype A vonden we sterk op elkaar lijkende virusstammen bij MSM. In genotype D, konden we twee onderverdelingen maken, één gerelateerd aan druggebruik en één gerelateerd aan de Marokkaanse bevolkingsgroep in Amsterdam. De overige stammen waren zeer verschillend en behoorden tot genotypen F, E and C. Tussen de 14 identieke stammen van MSM, was er één van een heterosexuele vrouw. Van de 14 identieke stammen in de groep "druggebruik" waren er zes afkomstig van heterosexuelen met wisselende contacten. In de groep bestaande uit twaalf stammen die gerelateerd leken aan overdracht in het buitenland of via buitenlanders, waren de overdrachtsvormen en virusstammen zeer verschillend. Uit deze bevindingen concluderen wij dat het Nederlandse HBV-preventiebeleid te kort schiet om overdracht van druggebruikers naar niet-druggebruikers te voorkomen en evenmin overdracht door chronisch geïnfecteerden uit endemische gebieden, voorkomt. Dit soort onderzoek, dat gegevens uit moderne microbiologische technieken koppelt aan epidemiologische gegevens van patiënten, levert noodzakelijke informatie voor preventiebeleid.

In HOOFDSTUK 6 richten we ons op hepatitis A, met een evaluatie van het preventiebeleid bij 1242 contacten van 568 aan de GG&GD gemelde gevallen in de periode 1996 tot en met 2000. De consensus in Nederland was om, bij melding van een geval van hepatitis A, de contacten op te sporen en op geleide van de immuun-status te beschermen met immunoglobuline (IgG). Vatbare (niet-immune) contacten, aan wie altijd IgG werd aangeboden, zijn zes weken later geïnterviewd en bij hen is weer serologisch onderzoek verricht. Hierdoor waren wij in staat om het beschermend effect van IgG te meten. Van 569 index patiënten konden we 1242 contacten opsporen. Meer dan de helft (672) was reeds immuun door een eerdere infectie, en bij 161 (28.2%) bleek een tegelijk met de index ontstane (concurrente) infectie te bestaan, waarvan 75 (47%) geen enkel ziekteverschijnsel aangaf. Van de vatbare contacten (409) die IgG kregen, kwamen er 186 (45%) voor heronderzoek na zes weken. Van hen bleken er 64 (34%) tussentijds geïnfecteerd, waarbij slechts 12 personen aangaven enige verschijnselen te hebben gehad, overeenkomend met 82% onbemerkte infecties. Bij navraag naar nieuwe ziektegevallen in de omgeving bleek er niemand anders meer ziek te zijn geworden (geen tertiare gevallen). IgG beschermt dus niet iedereen tegen infectie, leidt wel tot minder ziekteverschijnselen bij de geïnfecteerden, maar draagt wel bij aan verder terugdringen van de verspreiding. Mede op

basis van deze bevindingen is het preventiebeleid aangepast en wordt aan contacten liever vaccin gegeven dan IgG.

HOOFDSTUK 7 beschrijft opzet en resultaten van een proefonderzoek naar de haalbaarheid van virologisch onderzoek in faeces van nieuwe gevallen van hepatitis A in de jaren 1997/98. Van 33 personen, die aan de GG&GD Amsterdam gemeld zijn met een serologisch bevestigde acute hepatitis A, is materiaal verkregen, waarbij 8 personen meerdere monsters over een langere tijd na de ziekte hebben ingeleverd. Met een bijzondere laboratoriumtechniek (nested RT-PCR) gericht op een genetisch gebied van het virus, dat codeert voor de buitenste viruseiwitten (regio VP3-VP1 en VP1-P2a) is viraal RNA opgewerkt en onderling vergeleken aan de hand van de nucleotide-volgorde waarmee een afstammingsboom is gemaakt (phylogenetische analyse). Hiermee kunnen onderlinge overeenkomsten en verschillen tussen de aangetroffen HAV stammen en vermoedelijke relaties tussen patiënten zichtbaar worden. Bij 31 patiënten leverde de RT-PCR-test een positieve bevinding (genetisch virus-materiaal aangetoond), waarbij dat in 24 gevallen informatie opleverde voor beide genetische gebieden van het virus. Bij de patiënten die over een langere tijd faeces inleverden, was bij één tot de laatste dag van medewerking (dag 33) nog steeds viraal materiaal in de faeces aantoonbaar. De afstammingsboom liet zien dat er twee verschillende virussen rondgaan; één groep virusstammen (subgenotype 1B) bleek gerelateerd aan Marokkanen in Nederland, die duidelijk afweek van de virusstammen (type IA), die gezien werden bij mannen die sex hebben met mannen (MSM). Het onderzoek bleek haalbaar en leverde bruikbare gegevens over de verspreiding van HAV. Als, bij ruimere gegevensverzameling, bevestigd zou worden dat HAV subgenotype 1A zich in Nederland blijvend verspreid onder MSM, moet de vaccinatiestrategie daarop aangepast worden. Ook het vaccinatiebeleid bij kinderen van ouders die afkomstig zijn uit landen waar nog veel HAV voor komt, zou bij bredere gegevensverzameling op basis van nieuwe bevindingen aangepast moeten worden. Dit was voldoende reden een algemeen onderzoek onder alle gemelde HAV gevallen in Amsterdam in gang te zetten.

HOOFDSTUK 8 geeft de resultaten van twee jaar (2000-2002) gegevensverzameling en moleculair epidemiologische analyse van alle patiënten die bij de GG&GD gemeld werden met acute HAV. Van 104/156 gemelde gevallen (66%) kon viraal RNA verkregen worden voor nader phylogenetisch onderzoek. Net als in het proefonderzoek, werd weer geprobeerd twee genetische regio's op te werken, de nucleotide-volgorde te bepalen en te analyseren. De virusstammen bleken te passen bij drie genotypen: 1A, 1B and 3. Er waren twee onderling onafhankelijke verspreidingspatronen: in genotype 1A, zagen we vier groepen vi-

russtammen gerelateerd aan MSM, en een vijfde passend bij import uit het endemisch gebied Marokko. Eén van de MSM virusgroepen toonde gelijkenis met een Spaanse virusstam, waarbij een persoonlijke verbinding niet bewezen maar wel aannemelijk werd.
In genotype 1B, bleken alle stammen gerelateerd aan import, de meeste uit Marokko, al dan niet met verdere verspreiding in Nederland. In genotype 3, bleken de importgevallen afkomstig uit Pakistan. Vanwege de voortgaande verspreiding van HAV onder MSM via anonieme contacten, is de enige effectieve preventiemogelijkheid om alle MSM tegen HAV te vaccineren. Om import door kinderen te beperken, kan volstaan worden de reizende kinderen te vaccineren, maar hoeven niet alle Amsterdamse kinderen gevaccineerd te worden. Moleculaire epidemiologie biedt gedetailleerd inzicht in de feitelijke HAV-verspreiding en brengt onvermoede overdrachtscircuits aan het licht. Dit soort onderzoek kan nóg meer informatie opleveren als gegevens in Europees verband worden verzameld, zoals ook de European Working party on Food borne viruses propageert.

HOOFDSTUK 9 is een samenvatting van de bevindingen en geeft algemene conclusies uit de detailbevindingen. De GGD-benadering van de HBV-zwangerenscreening, waarin pasgeborenen en huisgenoten geïntegreerd worden benaderd, beschermd, en vervolgd blijkt een effectieve manier om veel risicopersonen te bereiken en te beschermen. Deze aanpak kan door alle GGD's overgenomen worden, en eventuele organisatorische varianten moeten zo snel mogelijk elders in Nederland met proefprojecten uitgeprobeerd worden. Drie studies zijn evaluaties van het GGD-handelen, die alledrie direct tot praktische beleidsaanbevelingen hebben geleid. Evaluatie hoort een vast onderdeel te zijn van alle GGD-interventies, niet alleen voor wat betreft HBV en HAV, maar voor alle vormen van infectieziektebestrijding. Dit is een onvoldoende belicht aspect van GGD-werk, waardoor inefficiënte interventies kunnen blijven bestaan en het kennisniveau in Nederland achter blijft.
Druggebruikers blijken een HBV-bron voor anderen, terwijl dit bij MSM binnen de groep blijft. Het is in het belang van de volksgezondheid om in de landelijke HBV-vaccinatiecampagne voor deze risicogroepen extra aandacht te besteden aan het beschermen van druggebruikers. Het proefproject resulteerde in een teleurstellend bereik in deze risicogroepen, maar de landelijke invoering kan veel profijt trekken uit de ontwikkelde benaderingsstrategieën. De landelijke aanpak maakt een betere berichtgeving en flexibiliteit in geografisch aanbod mogelijk, waar maximaal gebruik van moet worden gemaakt.
Voor wat betreft hepatitis A geldt dat vaccinatie van contacten beter is dan bescherming door IgG. Dit is inmiddels ook door de LCI overgenomen in het landelijk advies. Een kosten-effectiviteitsstudie moet uitwijzen of de voor de hand lig-

gende uitbreiding van de HBV-vaccinatie met HAV-vaccinatie voor MSM en kinderen met ouders uit HBV-endemische gebieden goedkoop genoeg is om in te voeren.
De molecular epidemiologische onderzoeken leveren bevindingen waar infectieziektebestrijding en –preventie direct gebruik van kunnen maken. De bevindingen zouden van doorslag gevende betekenis kunnen worden, voor algemeen beleid en bij individuele gevallen, als HBV en HAV uit heel Nederland op deze manier worden geanalyseerd; beter nog zou het zijn als dit in Europees verband gebeurt, zodat onvermoede overdrachtscircuits kunnen worden opgespoord en het percentage onbekende bronnen kan worden teruggedrongen.
Hepatitis B en hepatitis A zijn voorbeelden van infectieziekteproblemen die niet in één regio kunnen worden opgelost. Door de mobiliteit van de wereldbevolking zullen beide aandoeningen altijd een probleem blijven, zelfs in het rijkste, best georganiseerde land, totdat de HBV/HAV problematiek overal elders is opgelost. Het levert een bijdrage aan het terugdringen van de ziektelast in Nederland door in de derde wereld de gezondheidszorg van schone spuiten (HBV) en alle mensen van schoon drinkwater (HAV) te voorzien.

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